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University research in danger

POSTGRADUATE education comes in for some very rough treatment from the House of Commons Expenditure Committee in its recent report (96-I, HMSO; £0.34). If the committee had its way the numbers of those going straight from undergraduate education to any form of postgraduate work would be cut. 'Post-experience' students, however, would be encouraged both to take vocational courses and to do research for a higher degree. Maintenance for postgraduates would include a repayable loan element but tuition fees would not be required of British students. Overseas students, on the other hand, would be expected to pay the full cost of tuition (about £1,500) except insofar as reciprocal arrangements and overseas aid schemes could be devised.

The brief given to the Education and Arts Sub-Committee of the Expenditure Committee was to examine the "financing and administration of postgraduate education". It is important to remember this brief, because the committee clearly exceeded it in venturing into discussions on the character of postgraduate education. And, having strayed from its brief, it inevitably obtained incomplete evidence.

The nine members of parliament on the committee, none apparently with any firsthand experience of the sort of postgraduate study under discussion, heard evidence from most of the bodies concerned with universities and polytechnics and from many industrialists. It also travelled to seven other countries, where its witnesses were mostly concerned with the financing of students.

The committee, unsurprisingly, discovered that there are divided opinions about the value of higher education and doctoral research. It heard that industry, in general, cares little for the PhD and prefers to recruit at the graduate level. International Computers Limited said their needs for postgraduates were "negligible". The Confederation of British Industry (CBI) described a "widespread industrial view that the system is producing a body of specialists in science and technology, the relevance and originality of whose research is often questionable". This ill-considered remark, out of place in the committee's terms of reference, epitomises the woeful relationship between industry and most university departments. Industry views PhDs as poor material, PhDs view industry as a poor place for their skills.

Having unearthed this unhappy relationship, it would have been well to call for a much fuller enquiry. The committee should have recognised the problem and faced it head on with a much greater attempt to understand the world of postgraduate learning. This was not to be; there is no indication that the committee appre-

ciated why people do postgraduate research, what is unattractive in industry for the PhD and *vice versa*. Some of its foreign travel could have included attempts to find out why industrialists elsewhere do not share this contempt for the PhD.

The half-informed committee concluded that the solution to such problems as there obviously are is to let industry decide. Rather than question industry's miserable record in attracting and employing PhDs, the committee was content to go along with it in ripping the present system apart. "We see [postgraduate education] as specialised training for mature students who normally will have shown talent and determination well above average both in the academic and professional worlds". So the pre-experience student is to be discouraged, except from taking vocational courses.

If pre-experience study were restricted, would post-experience students grow in numbers? No doubt a demand could be created for vocational courses of up to a year. But would industry really release employees for three years to do a PhD? It does not specifically say that it would, indeed one major employer says privately that it would not. So the PhD would be a narrower qualification, dominated by those in the academic field and even less in tune with societal needs.

The government would do well to reject this poorly reasoned report, even though times are hard and relevance is the watchword. The structure of research training should not be pulled apart just because British industry and PhDs have not come to terms. What is good for the CBI may be appalling for the world of learning.

100 years ago



WE are glad to see that the *Times* has at last opened its pages to the question of the propriety of appointing a responsible Minister, whose duty it shall be to look after the interests of Science and of scientific research and education, and take charge of the scientific institutions of the country—institutions whose efficiency is at present sadly crippled from the want of a single responsible head. The whole question could not be better

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Bringing science and technology to the world's forgotten people

David Spurgeon, Director of Publications at the International Development Research Centre in Ottawa, discusses a unique approach, funded by the Canadian government, to applying science and technology to the problems of the Third World.

ONE of the most conspicuous ways in which science and technology have not lived up to their promise is in bettering the lives of the world's poorest people. The majority of these people (about 70%) live in the rural areas of some 100 developing nations, where the applications of science and technology have rarely penetrated. Large numbers of others leave the countryside seeking better conditions, but then find themselves residents of wretched squatter settlements surrounding metropolitan centres. Very little effort has been made even to try to apply science and technology directly to the problems of these, the world's forgotten people.

Robert S. McNamara, president of the World Bank, outlined the problem at the bank's annual meeting in Kenya last September:

"Despite a decade of unprecedented increase in the gross national product of the developing countries, the poorest segments of their population have received relatively little benefit," he said. "Nearly 800 million individuals—40% out of a total of 2,000 million—survive on incomes estimated (in US purchasing power) at 30 cents per day in conditions of malnutrition, illiteracy, and squalor. They are suffering poverty in the absolute sense."

In 1970, the Parliament of Canada passed an Act setting up a public corporation designed specifically to help such people. Called the International Development Research Centre (IDRC), its purpose is "to initiate, encourage, support and conduct research into the problems of the developing regions of the world and into the means for applying and adapting scientific, technical and other knowledge to the economic and social advancement of those regions. . . ." The brainchild of former Canadian Prime Minister Lester Pearson and UN Environmental Agency Executive Director Maurice Strong, the centre became the first agency of its kind.

Unique approach

What is unique in its approach is that, unlike most aid agencies, it provides funds for the scientists of developing countries to carry out their own research projects in areas of their own choosing, corresponding to their own national

priorities. At the same time, it attempts to build up research capability in these countries so that the indigenous problem-solving process can be perpetuated, and to promote regional communication in science and technology.

Last July the centre published a booklet called *The First 100 Projects*, which showed that in the 30 months from the inaugural board meeting in October 1970, to the meeting held in Bogota, Colombia, in March 1973, its board of governors had approved 100 projects costing \$13.4 million in some 60 countries. For the most part, these projects were concentrated on improving the well-being of rural people, and the agricultural projects were concentrated in the world's poorest areas, the semi-arid tropics.

The IDRC's funds are appropriated by the Parliament of Canada, but the Centre is governed by an international board whose members come from ten other countries as well, six of them developing countries. A small professional staff in Canada and at several regional offices around the world encourage applicants to formulate projects for approval by the governors, who also set the centre's policy. For administrative purposes, the projects are placed in four divisions: Agriculture, Food and Nutrition Sciences; Information Sciences; Population and Health Sciences; and Social Sciences and Human Resources.

An agricultural project that illustrates one approach the centre is taking to rural research can be found among small farms in the Andean foothills around Caqueza, east of Bogota, Colombia. The IDRC supplies funds to the Instituto Colombiano Agropecuario not just to increase crop and animal yields, but to improve the marketing system, provide farmers with easier access to agricultural credit and fertilizers, provide training for graduate students, professional staff and farmers and their wives, and supply a low-cost health service. The farmer himself is incorporated into the program: research is carried out on his land as well as on experimental farms.

Health and social change

The health of rural people is a concern of the Population and Health Sciences Division, which is seeking new ways of providing health services appropriate to the needs and resources of developing regions. New approaches to family planning are also being undertaken. But equally important are studies of the dynamics of population change: how and why populations grow or diminish in specific areas, what the influences are on fertility rates, and the social factors governing child-bearing decisions and decisions of people to migrate.

Understanding of the forces that produce change, the process by which change occurs, and its social, economic, political and cultural consequences are the goals of social science projects. One of the first of these was a study of slums and squatter areas in eight cities, from Lima through Lagos to Seoul, the reasons and the ways they grew. Another is a study of the role of hawkers and vendors, who play an important role in the marketing and distribution of agricultural produce in Asian cities. Still others are concerned with formation of technology policy.

Obviously, then, rural people are not the sole concern of the centre's programs. Owners of small industries are the clients, in another example—an industrial extension service set up in Southeast Asia which is designed to strengthen national technical information services already existing there. Specialized information centres (for example, on cassava, geotechnical engineering and irrigation) are also supported.

While it is too early to gauge the effects of all this activ-

ity, IDRC personnel have been gratified by the response they have received so far from those they work with in developing countries. Nevertheless, difficulties do arise, and one of the chief ones is a direct result of the centre's insistence upon scientists from the developing countries learning through their own mistakes. It is also a result of the heritage of an agrarian tradition, often intermixed with an overlay of colonialist attitudes from the past, and of present-day paternalistic practices of some more conventional aid programs.

Learning by doing

The difficulty lies in a lack of experience in research management in many developing countries. After so many years of having donor countries run their projects for them, developing country scientists, faced with the opportunity of doing their own thing, often do not know how to go about it.

Even formulating a proposal may be difficult, because someone else has always done it for them in the past, and because they think they must frame it in terms that will please the IDRC representative. Centre representatives sometimes have to say: "Look, it's *your* project. How do *you* want to handle it?"

Such difficulties mean a loss of research time. One project lagged for months while an African country looked for ways of buying equipment in foreign currency without converting its own currency rules, which forbade sequestering of foreign funds. Yet IDRC officials remain convinced that developing countries should, as far as possible, be left to work out such problems for themselves, because this is an essential part of the learning process. "I'd rather let a project lag a year behind than go in and tell them how to do it", says one director. "I am willing to sacrifice some quality of research for the experience gained in the process of doing research", says another.

Such an attitude is not always appreciated by those in developing countries. If their scientists have worked with other donor agencies, they may feel a lot of time could be saved if routine matters like supplying equipment could be handled for them. And their politicians sometimes want technical experts from developed countries sent in because they want to see results in a hurry. The IDRC only provides experts when local trained persons are impossible to find in a recipient country, and when the country asks for it—and even then they work as advisers with the project staff, rather than telling the local scientists what to do.

A distinction can be made between the management of a development project as a whole and the management of scientific research. Both seem to pose problems in developing countries. IDRC directors claim that, when a development project fails, it is rarely for lack of will, resources, or knowledge, but for lack of management capability. The difficulty lies in putting all the pieces of the puzzle together.

In research management, the problem is not only one of identifying problems and agreeing on goals, but also one of logistics. One laboratory in southeast Asia, for example, was found to have 50 years' supply of glass tubing in one size but none whatever in any other size.

National coordination

On a national level, a general lack of coordination of development activity exists, although Malaysia, for example, has achieved success by adopting a sort of "war-room" approach, observing the progress and interaction of the whole

attack on development from the point of view of a single operation, as was done in war-time with military activities. The Philippines have adopted a similar systems engineering approach.

In outlining these problems of developing countries, I do not wish to suggest that the so-called developed nations have all the answers. Far from it. We can hardly expect developing nations to agree on national goals and coordination of research when we have not been able to do so ourselves. And that is surely the case, as a study of recent science policy literature will amply confirm.

Nevertheless, the IDRC is trying to do something about such problems, and indeed already has had some success. Centre funds are being used increasingly to bring scientists from developing countries together to talk about their mutual problems, and a workshop is being sponsored to encourage education in research management and to assist in identification of problems.

One meeting funded by the centre brought together nine senior scientists from as many different countries, no two of whom had ever met before. A result of this meeting was the establishment of a course in post-harvest technology of rice for middle-level managers in rice processing plants. A number of similar courses were later set up elsewhere. Funding was really all the centre provided in this case: the scientists themselves identified their own problems and ran the meeting.

In another instance, the centre encouraged the agricultural credit bank in an Asian country to provide revolving credit for local farmers by the simple expedient of setting an example by first doing so itself. Credit had never before been available to these farmers, but when they and the bank saw what it could do for them, the bank followed suit.

A question of balance

Not all types of projects are equally popular in the Third World. The benefits of an agricultural or a health delivery scheme are fairly obvious, and many countries make proposals in these areas. But the value of, say, a computerized data base or agricultural research information system is harder to envision for traditional societies, despite their enormous potential. Apart from anything else, developing countries are far more likely to have agricultural or medical experts of their own than information scientists.

In a statement to the board of governors' annual meeting last March, IDRC President David Hopper identified eleven policy issues in the centre's novel approach to foreign aid. All involved a question of balance. For example: How much should the centre follow the priorities of developing nations, and how much should it exercise its own judgment as to the "proper" priorities for development? How much should it emphasize the training aspect of research at the expense of first-class results? What should be the balance between support for research and direct support for post-secondary or postgraduate training, particularly in countries where training is vitally needed? What should be the balance between problem-oriented or applied research (so far emphasized by the centre), and phenomenon-oriented or basic research (now obviously necessary in some areas before further progress can be made)?

Perhaps the greatest test of the impact of the IDRC's approach to the field of foreign aid will be the influence it exerts on other international agencies to imitate its style of operation. There is evidence that these agencies are watching the experiment with more than usual interest.

international news

Defence, energy and Keynes shape US Science budget

Colin Norman, Washington

MEMORIES of the golden days of the early 1960s, when research budgets in the United States were lush with money, were carefully revived this week by President Nixon's budget proposals for the 1975 fiscal year. A massive increase in funding for the federal government's research and development programmes has been proposed—if it is all spent, it will be the largest single increase in funding for science and technology in more than a decade.

Unfortunately, however, there is absolutely no guarantee that the money will be spent. And, in any case, a careful look at the budget figures suggests that the overall prospect for research and development is not as rosy as it appears at first sight. In fact, some programmes will actually be cut back, and the increases being proposed for many others are insufficient to keep pace with inflation—particularly if it continues at its present galloping rate.

The budget—a collection of figures, facts and promises, richly laced with rhetoric—has been sent to Congress where it will be chewed over by the appropriations committees. The figures should be treated with extreme caution, for they are far from being money in the bank. For one thing, the spending proposals can, in theory, be drastically altered by Congress. And, for another, even if Congress agrees to the proposals, the Administration may later refuse to spend the money. It was, after all, only two years ago that President Nixon proposed large increases in spending on research and development, but ended up by impounding several hundred million dollars in order to stave off inflation. Nevertheless, the importance of the budget is that it provides a good indication of the Administration's overall policies for science and technology.

The sharp increase proposed for federal research and development owes its existence to three chief factors: a staggering increase in the budget for de-

fence-related science and technology; an equally spectacular jump in the proposed outlays on energy research and development; and the Administration's partial espousal of the teachings of John Maynard Keynes—in an effort to head off a general recession, an expansionist budget has been proposed and science and technology will get a share of the largesse.

The overall strategy for research and development outlined in the budget is to increase expenditures on programmes likely to help solve pressing domestic problems—such as energy research and development—and to pump up the defence budget. But, on the other hand, expenditures in most other areas will be held back.

Part of the story is contained in Table 1. The overall increase in obligations for science and technology is a massive

\$1,626 million—up from \$17,930 million this year to \$19,556 million in 1975. (Obligations represent budgetary commitments, which may involve expenditures spread over several years.) But defence-related research and development alone is set to increase by more than \$1,000 million, and energy research and development is earmarked for an increase of \$816 million—from \$999 million this year to \$1,815 million next. Simple addition shows that the combined increase for those two areas is greater than the total increase for all federally sponsored research and development. Some programmes are thus clearly being cut to accommodate them.

One area to suffer is space technology—NASA's space budget will decrease by almost \$200 million, chiefly because of the end of the Apollo and Skylab programmes. The research and development

TABLE 1 Conduct of Research and Development (in millions of dollars)

Department or agency	Obligations			Expenditures		
	1973 actual	1974 estimate	1975 estimate	1973 actual	1974 estimate	1975 estimate
Defense—Military functions.....	8,382	8,573	9,581	8,417	8,676	9,201
National Aeronautics and Space Administration.....	3,085	3,309	3,122	3,271	3,104	3,173
Health, Education and Welfare.....	1,844	2,332	2,228	1,791	2,191	2,354
Atomic Energy Commission.....	1,361	1,429	1,702	1,361	1,429	1,709
National Science Foundation.....	480	530	654	428	460	538
Transportation.....	311	358	396	312	342	364
Agriculture.....	371	393	412	349	389	416
Interior.....	254	287	510	235	282	428
Commerce.....	191	210	266	179	192	233
Environmental Protection Agency.....	181	174	336	145	180	264
Veterans Administration.....	74	85	94	75	85	94
Housing and Urban Development.....	58	65	70	48	58	67
Justice.....	35	52	56	24	46	56
All other.....	176	132	128	150	151	125
Total, conduct of research and development ¹	16,802	17,930	19,556	16,784	17,585	19,016
Total, conduct of research.....	6,478	7,287	7,607	6,428	6,971	7,483
Total, conduct of development.....	10,324	10,643	11,950	10,356	10,613	11,532

¹ Details may not add to totals due to rounding.

activities of the Department of Health, Education and Welfare also appear to have suffered a cutback, but the picture there is clouded by the fact that the 1974 figure is swollen because it included funds held over from 1973. Other areas, with a few notable exceptions, are being held constant.

Asked last week whether he believes that the money proposed for research and development outside the energy and defence areas is sufficient, Dr H. Guyford Stever, Science Adviser and Director of the National Science Foundation, said that it represents a correct ordering of priorities for this year. In a press briefing, called to explain the budget for research and development, Stever claimed that civilian science and technology will enjoy an increase of nearly \$1,000 million under the Administration's budget proposals. The proportion of the federal research and development budget devoted to defence and space research will thus drop from 68% to a mere 65%, continuing the trend established during the past few years (see Fig. 1).

By far the greatest increases will come at the development end of the research and development spectrum—a fact which is consistent with the emphasis on defence and energy research and development. Spending on research is set to increase by \$320 million, from \$7,287 million to \$7,607 million. At just over 4%, that is clearly insufficient to keep pace with inflation. As for research and development in colleges and universities, expenditures are set to go up by about 7% under the budget proposals, with much of the increase coming from the National Science Foundation.

Overall, the prospects for research and development held out by the budget look bright. But what are the chances that Congress will go along with the proposals? As far as energy is concerned, it can safely be assumed that in an election year, and with the energy crisis constantly in the headlines, Congress will be unwilling to cut budget requests aimed at easing the energy shortages—in fact, the reverse is probably true. In other areas, however, the budgetary prospects are more difficult to predict.

The way in which the budget proposals would affect various branches of science, and individual government agencies, is discussed in more detail below.

Energy Research and development. Reporters attending the press briefing given by Dr Stever were handed three pieces of paper containing details of the budget proposals for energy research and development. The details differed substantially from one paper to an-

TABLE 2 Research and Development in Colleges and Universities (in millions of dollars)

Department or agency	Expenditures		
	1973 actual	1974 estimate	1975 estimate
Health, Education and Welfare.....	937	1,159	1,232
National Science Foundation.....	329	339	427
Defense—Military functions.....	218	204	203
National Aeronautics and Space Administration.....	127	97	91
Atomic Energy Commission.....	83	84	91
Agriculture.....	88	95	99
All other.....	106	128	136
Total.....	1,888	2,107	2,283

other, a fact which not only added to the general obfuscation, but which also bears witness to the extreme haste with which the energy research and development proposals were thrown together. The final amounts were, in fact, settled only a few days before the budget was sent to Congress, and appeared as an appendix to the main budget proposals.

The chief outlines of the energy research and development proposals were revealed by President Nixon in January (see *Nature*, 247, 247; 1974), and although some of the numbers have changed slightly since then, the overall thrust has not been altered at all. In short, an increase of 81% has been proposed for energy research and development, with coal research singled out for a particularly large increase. Nuclear fission is set to carry off about 40% of the \$1,815 million that has been budgeted for all energy research, and solar and geothermal energy are earmarked for large increases.

The objective of the energy research and development programme is to exploit domestic energy resources as rapidly as possible, in order to offset imports of oil from the Middle East. The

project has been given the name Project Independence by President Nixon, who has vaguely defined its goal as creating a capacity for energy self sufficiency in the United States by 1980. Last week, however, John Sawhill, Deputy Director of the Federal Energy Office, outlined just as vague, but rather more modest (and realistic) an aim for Project Independence. "By 1980", he said, "we want to demonstrate to the rest of the world that we are well on the way to energy self-sufficiency".

National Defence. Although the United States is no longer fighting a war, and although the number of men in the armed services has been reduced, the budget request for the Department of Defense is a record \$87,700 million, an increase of more than \$7,000 million over this year's defence budget. Within that total, the amount proposed for research and development is set to increase from \$8,573 million to \$9,581 million. In addition, the Atomic Energy Commission is proposing to spend \$631 million on defence-related activities, an increase of \$24 million over this year's outlays.

The budget documents are characteristically vague about how this massive amount of cash will be spent, and Dr Stever, whose brief as Science Adviser does not include the defence half of the research and development budget, could only offer the information that about \$80 million falls into the category of research, while the rest is labelled development. The Department of Defense is proposing to spend about \$203 million of its research and development budget in the universities—about the same amount as in the past two years.

Biomedical research. Once again, the politically sensitive areas of cancer research and research into the causes and prevention of cardiovascular and pulmonary diseases have been favoured by large budget increases, at least partly at the expense of other areas of biomedical research. The Administration's

Conduct of Research and Development—Obligations

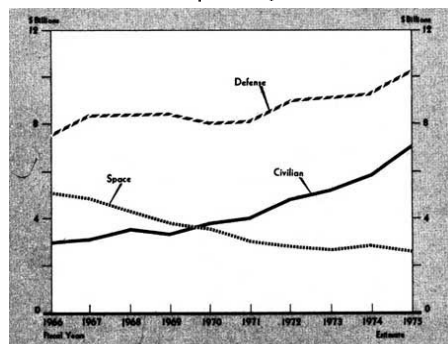


FIG. 1 Conduct of research and development—obligations.

budget request includes \$600 million for the National Cancer Institute (NCI), which represents an increase of \$73 million over this year's budget, and \$309 million for the National Heart and Lung Institute (NHLI)—an increase of \$22 million. The combined budgets of all the other institutes at the National Institutes of Health would, however, remain static. In other words, if inflation is taken into account, all the institutes except the National Cancer Institute and the National Heart and Lung Institute will suffer budget cuts if the Administration's spending proposals are agreed to by Congress.

The picture is, however, slightly distorted by the fact that the Office of Management and Budget impounded large amounts of money earmarked for NIH in 1973, but released it in 1974, thus swelling the 1974 totals. A slightly clearer picture can be gleaned from the proposed expenditure levels in 1975 (as opposed to obligations), which are set to increase for every institute. Nevertheless, the 1975 budgets for all the institutes, again with the exception of the NCI and NHLI, are lower than the budgets in 1973.

Last year, when the Administration's spending proposals for biomedical research were announced, they provoked an outcry in the biomedical research community over the plan to scrap the NIH training and fellowship programmes. Under strong pressure from Congress, Caspar Weinberger, Secretary of Health, Education and Welfare, announced in June last year a revamped NIH fellowship programme, to support postdoctoral trainees in priority areas of biomedical research. The new programme was given \$27.5 million this year—enough to support 1,825 fellowships—and it is set to get a further \$27.5 million in 1975 under the Administration's budget plan. Those amounts are, however, much less than Congress has been pushing for, and thus the fight over biomedical training seems far from finished.

Another major change being proposed by the Administration in 1975 is the phasing out of the General Research Support Program, a programme funded from NIH's Division of Research Resources, which provides general building support to institutions in proportion to the amount of biomedical research funds they receive from NIH. The programme is being phased out, according to the Department of Health, Education and Welfare, because "the institutions receiving this general support are now among the strongest research institutions in the world".

National Science Foundation. Like

most of the agencies on the federal research landscape, the National Science Foundation has been selected to share in the large increases in funding proposed for energy research and development. Its total budget is set to increase from \$646.4 million this year to \$788.2 million next year, a jump of \$141.8 million. Of that increase, however, \$133.4 million is for energy related programmes, thus the rest of the NSF budget is what Dr Stever calls "tight".

Nevertheless, the NSF's programmes in support of basic research fare particularly well. The scientific research project support programmes, for example, are earmarked for an increase of some \$72.4 million (of which \$53.6 million is for energy related programmes) and will reach a total of \$363.7 million if the Administration's budget proposals are accepted by Congress. The budgets for the national research centres are also set to increase, from \$42.5 million to \$52.5 million, with 13 million included in the budget of the National Radio Astronomy Observatory for construction of the Very Large Array telescope system.

NSF's programme of Research Applied to National Needs (RANN), an applied science programme which has suffered mixed fortunes at the hands of Congress, is set for a large increase in funds, from \$73.8 million to \$148.9 million—all of the increase being soaked up by energy research and development. Within that total, an extra \$36.8 million will be heaped into solar energy research, and \$16.6 million is earmarked for research on geothermal energy.

To accommodate some of the increases in energy research funding, some programmes have, however, suffered cuts. Most prominent casualty is the Experimental Research and Development Incentive Programme, an effort begun just two years ago which was aimed at testing methods for encouraging innovation in technology-based industry. The programme is being slashed by \$11.3 million, and will move, according to Dr Stever, "into an evaluation mode". That seems to be a euphemism for the programme's termination, for it is left with only about \$1 million in 1975.

Space research. Although the National Aeronautics and Space Administration is again hard pressed for money, space science has fared relatively well. The Venus Pioneer programme has been given the green light, and a powerful new infrared telescope is to be built on Mauna Kea in Hawaii.

NASA officials have managed to survive this year's budgetary battles without losing any major programmes—the

first time for several years. Nevertheless, the agency's budget is still well below the \$3,500 million level which Dr James Fletcher, NASA Administrator, said last year is needed for a balanced programme. Chief casualty of the financial stringency is the space shuttle, whose development schedule has slipped by about six months—the first manned orbital flight is now expected to take place in mid-1979 instead of towards the end of 1978. Fletcher said last week that he has obtained assurance from OMB, however, that there will be no further slippage in shuttle development.

The Administration's budget includes \$27 million for the Venus Pioneer programme. The idea is to fly two missions to Venus in 1978, one of which will send four entry probes into the Venusian atmosphere, and the other will place a spacecraft in orbit around the planet. Both missions will be launched by Atlas-Centaur rockets, which is a change from original plans to use Delta launch vehicles. NASA found that the savings from removing some weight constraints outweighed the extra costs of the launch vehicles.

As for the infrared telescope, it will eventually cost some \$6 million. It will be a 3 m telescope, located 14,000 feet above sea level, and its chief use initially will be to provide data on Jupiter and Saturn necessary for planning the Mariner Jupiter-Saturn flyby mission which is due to be launched in 1976.

Other notable features in the NASA budget are that the go-head has been given for SEASAT, a satellite which will measure the physical characteristics of the oceans; launch is set for 1978. The ERTS-B satellite has been rescheduled and will now be launched early in 1975—a year earlier than planned.

High Energy Physics. Once again, the 200 BeV accelerator at National Accelerator Laboratory at Batavia, Illinois, is being given a large increase in operating funds, largely at the expense of the other particle accelerators. The 200 BeV machine is due to get \$36 million in 1975, an increase of about \$7.6 million. Meanwhile the Alternating Gradient Synchrotron is set for a modest increase of \$240,000 and the Stanford Linear Accelerator will get an extra \$400,000, the Zero Gradient Synchrotron is being cut by \$600,000, and the Bevatron will suffer a cut from \$4.2 million to \$1.7 million. According to a spokesman for the Atomic Energy Commission, however, the drastically reduced funding for the Bevatron does not indicate that the AEC is closing it down—it will, in fact pick up some extra funding from other parts of the AEC budget.

Bright ideas

John Hall

A PLEA for long term plans to use solar energy rather than entering a "Faustian bargain" to employ a nuclear technology was made by Professor D. Bryce-Smith (Department of Organic Chemistry, University of Reading) at the inaugural meeting of the United Kingdom section of the International Solar Energy Society in London recently. Professor Bryce-Smith urged a change in the climate (of opinion) so that short term economic considerations which dictated a nuclear strategy should not prevent the investment of money in a long term energy solution based on the power of the Sun. It was an appeal calculated to meet with immediate approval, since it was delivered to a meeting which filled the lecture theatre of the Royal Institution to overflowing with devotees of the solar economy.

What the faithful witnessed, apart from this brief obloquy against a nuclear power programme, was a quartet of lectures on solar possibilities, nicely illustrated with a slide show, and a clutch of demonstrations managed by Professor Sir George Porter in the best theatrical traditions of the institution. The only spontaneous round of applause during the entire meeting was accorded to a model railway engine which obligingly described a circuit whenever a bright light was directed on to its battery of photovoltaic cells. Alas, a further setpiece, meant to illustrate how light energy might be stored by a reversible process of chemical change, failed to function on account of technicality, and though belief in the theory can scarcely have been suspended, in many minds there was a distinct sense of pique at having been cheated of a spectacle. (Why is an elementary demonstration of a footling principle received like a virtuoso performance of conjuring by an assembly specifically equipped to see the simplicity of the feat? That is show-business.)

Gadgetry apart, Sir George made the point that although photochemical energy conversion might be a going concern in the field of space engineering, it is distinctly unpractical on economic grounds for generating power on *terra firma*. Apparently, the cost of making a 1 kw plant, using pure silicon crystals, is something in the region of £1,600. Polycrystals make the proposition cheaper, and metallurgical silicon is capable of offering a rate of £2 per kw of output. But given this cost, and the facts that the most efficient cell of this kind only converts 14% of the solar energy falling on it, and that this is difficult to store, a good deal of work needs to be done before photochemical

energy conversion can make a sizeable contribution to the economy.

Professor J. K. Page (Department of Building Sciences, University of Sheffield) also made the point that the central research issue in solar energy is the question of storage. Speaking about the architectural requirements of successful solar usage, Professor Page pointed out that when we really want energy, in winter, the Sun does not offer all that much; and in summer we do not want all that is available.

He also, however, indicated that any reservations one might harbour about the unreliability of British summers is not in itself an argument against our benefiting from solar radiation, since direct sunlight is not essential to energy conversion; diffuse energy is available throughout the year and could be used in various ways. But, Professor Page suggested, perhaps the first priority is to conserve rather than harness the energy actually falling on the Earth. (In Australia in one year the amount of energy arriving from the Sun is equivalent to the total of the planet's reserves of fossil fuels, so they say.) The need, then, is to build houses which let in the heat when it is around and hang on to it as long as possible.

In the same area of bending the mind on comparisons of solar and conventional energy sources, Dr D. O. Hall (King's College, London) contended that the amount of biologically useful solar energy falling on the Earth in three days is equivalent, yes, to the total of the planet's proven energy reserves. Concentrating on photobiological energy conversion, Dr Hall calculated that if the sunlight falling on the United States in one year could be converted at 10% efficiency, only 1.5% of the land area would have been sufficient to provide the country's total energy requirements for 1970; and meanwhile, back in Australia, only about 0.03% of the land mass could have supplied the nation's needs.

Naturally, an obstacle to the realisation of this economic miracle is the harvesting and burning of the energy-rich products of photosynthesis. It would be a big job. Among priorities to improve the utilisation of solar energy by biological means, Dr Hall suggested the fermentation and pyrolysis of plant material to produce liquid fuels and gases; genetic engineering to produce plants which are more efficient as converters by photosynthesis, or plants which might be made to produce fatty acids or proteins; and the direct production of hydrogen by means of a photosynthetic system using water as the electron donor.

The ready production of cheap hydrogen (Dr Hill has not been the first to observe) would herald a new age of energy usage. Hydrogen obtained by

photobiological means would use an inexhaustible supply of energy (solar) and an inexhaustible supply of reagent (water) to produce a non-polluting energy. What price the new millenium?

Nader embarrasses the US AEC

Colin Norman, Washington

CRITICS of nuclear power in the United States have been engaged in the past two weeks in a brutal public battle with officials of the US Atomic Energy Commission over the safety of American light water reactors. The battle took place in the committee room of the Joint Committee on Atomic Energy, whose members were supposed to be holding the ring but ended up weighing in heavily on the side of the AEC. The charges and countercharges traded during the hearings are by now familiar stuff, but at least one attack grabbed headlines and put the AEC publicly on the defensive.

Ralph Nader, scourge of the automobile industry and consumer advocate *par excellence*, launched a blistering attack on the joint committee itself because "its zeal for promoting nuclear power has so overshadowed its responsibility to assure public safety that it has put at risk not only this generation, but a hundred generations to come". Nader also released an internal AEC task force report on the safety of nuclear power plants which, he said, shows that AEC officials have been "misrepresenting the facts" when they have assured the public of the safety of nuclear reactors.

AEC officials responded with some embarrassment to the disclosure of the task force report, suggesting that Nader had an early draft and that he was drawing false conclusions from the material it contained. Their response was to polish the report up and get it into the public domain as quickly as possible. They hope to publish a final version of the report sometime this week.

Nevertheless, Nader clearly scored points by unveiling the report in this manner. The task force, Nader said, "does not believe that there is the required confidence level that the probability for (a major) accident is one in a million or less per reactor year". Just a few days before Nader made his statement, Dr Dixy Lee Ray, the Chairman of the AEC, had indeed put forward the one-in-a-million suggestion as the basis for her contention that nuclear power plants are safer than most other forms of power generators.

The task force, Nader said, "assembled the most disturbing evidence of safety problems at US nuclear power

reactors and documented the superficiality and incompetence of AEC licensing of nuclear power plants". Quoting directly from the report, Nader said that safety problems are "besieging" American nuclear power plants, and the level of safety of such power plants is "an unanswered question".

Most of the detailed examples of safety problems cited by Nader involve engineering defects which came to light after the plants were built. For example, he quoted a finding of the task force that five plants built by General Electric Company had, among other defects, broken hangar bolts, no lock nuts and improper bolts, and the deficiencies "could negate operability of the ECCS (the emergency core cooling system, which is supposed to flood the reactor core with water if the primary coolant is lost in an accident)".

Nader ended up by suggesting that the joint committee should dissolve itself because of its poor track record. But

that suggestion did not go down too well with the members of that august body, and a verbal battle broke out. In particular, Mike McCormack, who was a nuclear chemist before becoming a politician, said that Nader's statements "revealed very profound ignorance of the whole subject".

Meanwhile, in Britain, the former General Manager of the US Atomic Energy Commission, Professor C. I. Wilson, listed the hazards of American light water reactors, in order of importance. First and foremost, he said in an interview with the *Times*, are doubts about the inherent safety of the light water pressure vessel containing the nuclear core, which could melt into "a radioactive puddle" in the event of a failure. Reactors of this type, he said, were "most unattractive animals to have as neighbors". And, if they really have to be built, he would rather see them 500 feet underground, in a sound geological formation.

An adviser on energy to the United States government, and a faculty member of the Massachusetts Institute of Technology, Professor Wilson went on to voice concern about nuclear reactors in general. After pressure vessel failure, the second most alarming prospect in his book is the production of large quantities of highly toxic material. The technology for handling the plutonium generated in breeder reactors is in its infancy, Professor Wilson said. Plutonium is the most toxic material known: AEC standards allow only half of one millionth of a gram to be absorbed by a person in a lifetime, but reactors are producing as much as 250 kg of plutonium each.

He is also anxious about the safety of future generations who are being left with a legacy of stored radioactive fission products. Although, he added, he has a degree of confidence that long term handling of the waste can be arranged safely.

correspondence

Energy strategy

SIR,—I am sorry that travel has kept me from responding earlier to your provocative leader 'A Broader Debate on Energy' (*Nature*, **246**, 179; 1973).

In kindly discussing my monograph, 'World Energy Strategies: Facts, Issues, and Options', you attempt what I did not dare—a brief summary of an extremely complex argument—and thereby, I fear, distort its thrust and substance.

I do not "see coal as the short and medium term solution to the energy problem", but as part of a diverse strategy stressing conservation and "unconventional" sources. Much better ways to mine, burn, and convert coal can help us to bridge to a sustainable energy economy, but are not a panacea.

You suggest that the paper ignores the social costs of coal. Careful reading does not support your view. Full weight is given to these high costs, which can be reduced but not eliminated. My "naïve reference to a . . . deep mine which 'is said to use every kind of . . . precaution yet is still profitable'" is not an attempt to "let (coal) off the social hook", but a criticism of many operators for refusing, on economic grounds, to enforce precautions already known.

You refer to my "biased point of view . . . prepared to give coal the benefit of every doubt, nuclear energy the

benefit of none". In fact, when all I knew about fission was what its proponents told me, I welcomed fission as a 'clean' alternative to coal. I am now prepared to give fission technologists full marks for zeal and cleverness, partial (and, in the USA, often undeserved) marks for diligence, and zero marks for recognising crucial gaps between intention and performance.

Many scientists far more distinguished than I are coming to appreciate the impact of human fallibility on a technology in which "no acts of God can be permitted" (Alfvén). Pugwash/Aulanko is one sign of a profound shift of opinion now underway. Another: last autumn, of 20-odd senior energy experts whom I saw in Washington, all were worried by fission and frightened by fast breeders; two-thirds wanted to go promptly to a fission-free economy. A year earlier, nearly all had been enthusiastic fission advocates.

A summary of recent convergence amongst sophisticated energy strategists cannot shrink from heterodoxy. It must attempt a thoughtful analysis of safeguards, safety, and waste disposal as they might be practised in the real world, not merely as they are designed in blueprints. References to Frisch's familiar paper are no substitute for the careful study that these issues deserve.

I could not agree more with your call for broad, holistic, up-to-date public

and professional debate on Britain's long-term energy future. I am sure that many will join me in offering our help if you, Sir, are prepared to use your influence in practical initiatives to this end.

Yours faithfully,

AMORY B. LOVINS

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Bomb disposal

SIR,—Mr Davidson's suggestion of using liquid nitrogen for bomb disposal purposes (*Nature*, **246**, 548; 1973) is a technique invented by the Royal Engineers thirty years ago. The Germans had developed their 'Y' fuse which used mercury switches as an anti-handling device. The counter-tactic developed was to freeze the mercury in the fuse and then remove and dispose of the device. Liquid nitrogen, liquid oxygen, and a mixture of solid CO₂ and methanol were all used as refrigerants. The technique was completely successful. Plus ça change. . . .

Yours faithfully,

P. R. W. WEBB

*Office of Science and Technology,
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news and views

X-ray sources and Occam's razor

WHEN two or more theories are available to explain the same observable phenomenon, it is usually a good bet to accept the simplest theory as the basis for further investigation. This powerful rule of thumb has many applications in astronomy, and 'works', for example, if used to assess the relative merits of theories of planetary motions. Straight-forward elliptical orbits are simpler and easier to work with than complex interactions of cycles and epicycles, although admittedly the second model can be made to give the right answers. So can this philosophy be applied to the question of black holes, which is currently one of the most contentious issues in astronomy? Clearly, it can; the question which must be resolved is whether it is simpler to explain observations of objects such as Cyg X-1 in terms of black holes, or by invoking more conventional astronomical phenomena.

There is no doubt that the rapidly varying X-ray sources which burst upon the astronomical scene with the advent of the Uhuru satellite must be very compact objects. The only plausible energy source for the X-ray production mechanism is the release of gravitational energy as matter accretes onto a small, dense object—that is, a white dwarf, a neutron star or a black hole. Furthermore, the lengths of the periodicities found in some of these sources suggest, from even the most naive considerations, that the X-ray flux observed is being modulated by rotation or pulsation of just such compact objects. Perhaps it is natural that theoreticians should look for explanations involving the more extreme of these alternatives, since it is only by studying events occurring in such extreme conditions that theories can be pushed beyond their present limits of known reliability; however, there has recently been a tendency for models of these X-ray sources to move back one step towards the less exotic. In the case of Her X-1 and Cen X-3, it seems that white dwarfs, rather than neutron stars, are sufficient to explain the observations; in the case of Cyg X-1, a plausible model involving a neutron star rather than a black hole is put forward on page 351 of this issue of *Nature* by Fabian, Pringle and Whelan.

As far as Cen X-3 is concerned, the first suggestion that the periodic variations found could be explained in terms of white dwarf pulsations came very soon after the discovery of these periodicities (Gribbin, *Nature, phys. Sci.*, **233**, 18–19; 1971). But although that model "has the merit of providing a ready explanation for the fluctuations in the periods . . . which could be related to changes in the property of the star caused by the impingement of . . . gas streams", in 1971 attention remained focussed on neutron star models, and few people wanted to know about mundane explanations in terms of white dwarfs. Recently, however, DeGregoria and Woltjer have taken this idea a step further (*Nature phys. Sci.*, **246**, 108–109; 1973). They have investigated both Her X-1, which has a period of roughly 1.24 s that varies in a "rather complex" way, and Cen X-3, which shows a roughly 4.8 s period. It seems that certain models of pulsating white dwarfs which failed to account for the pulsar phenomenon can explain these X-ray variables. The mass inferred for Her X-1 is $1.3 M_{\odot}$, and DeGregoria and Woltjer point out that the 1.24 s period of the source corresponds

to the first harmonic vibration of a pure helium white dwarf model with a mass of $1.2 M_{\odot}$, according to Faulkner and Gribbin (*Nature*, **218**, 734–736; 1968); similarly, the 4.8 s variation of Cen X-3 can be explained in terms of a white dwarf of mass $0.6 M_{\odot}$.

One powerful argument in favour of these models is that "no plausible evolutionary scheme exists to explain the presence of a neutron star in . . . HZ Her" but "many binaries with white dwarf companions do exist" and there is no reason why mass transfer in these systems should not lead to X-ray production. "Models involving pulsating white dwarfs should not be prematurely rejected", say DeGregoria and Woltjer.

The model for Cyg X-1 put forward by Fabian *et al.* is a little more exotic than these white dwarf models—but less exotic, perhaps, than models involving black holes. There seems little doubt that if Cyg X-1 is a binary then the X-ray emission is associated with a compact object so massive that it must be a black hole. But is Cyg X-1 a binary? About a third of observed binaries are actually triple systems, and if Cyg X-1 is one of these then the mass problem can be removed. If the secondary is itself made up of two stars, a $1 M_{\odot}$ neutron star orbiting a $10 M_{\odot}$ B1V star, the observed modulation of the light curve of Cyg X-1 can be explained.

The conclusion is that "as yet there is no need to invoke the existence of black holes to explain the properties of X-ray sources". That may come as a disappointment to those relativists still searching for a spectacular proof that general relativity holds even in extreme conditions; but the mere suggestion that black holes might have been discovered has produced a wealth of theoretical ideas encouraging rapid development of a better understanding of high energy astrophysical processes, acting as a catalyst in a way reminiscent of how the steady state theory encouraged the development of cosmology in the 1950s and 1960s.

It may be that when the impossible has been eliminated whatever remains, however improbable, must be the truth. But alternatives to the black hole explanation of Cyg X-1 are not, it seems, impossible after all. J. G.

Cysteines at the interface

ONE of the most intriguing aspects of haemoglobin chemistry has been waiting more than a century for an explanation in structural terms. Since its discovery by Körber in 1866, the striking resistance of human foetal oxyhaemoglobin (Hb-F) to alkaline denaturation, in comparison to the adult protein (Hb-A), has been exploited by clinical chemists for the analysis of mixtures of Hb-F and Hb-A. It is practicable to work at a pH, around 12.6, where the times for half-denaturation differ by two orders of magnitude (about 1000 s for Hb-F compared with about 10 s for Hb-A), and much ingenuity has been shown in devising convenient routine procedures and in extending the sensitivity and reliability of the methods to low proportions of Hb-F.

It has often been suggested that this difference in stability of Hb-F and Hb-A arises from the ease by which the tetramer molecule dissociates into dimers and monomers. Since the work of Itano and colleagues in 1967 and of others, the

higher resistance of Hb-F to dissociation at both low and high pH has been well established. The present knowledge of the tertiary and quaternary structure of haemoglobin, and of the differences in the amino-acid sequences of the polypeptide chains of a large number of haemoglobin species, has now been used by Perutz (see page 341 of this issue) to provide an explanation for the large variations in their rates of alkaline denaturation.

In accordance with earlier views that the rate-limiting step is likely to be the formation of monomeric subunits from the dimer, Perutz has looked at sequence differences located at the $\alpha_1\beta_1$ interface for evidence in support of his hypothesis that denaturation by alkali is activated by the ionization of buried, weakly acidic side chains. Comparing Hb-A with bovine haemoglobin, which is almost indefinitely stable at pH 12.7, Perutz concludes that the significant structural difference between these two haemoglobin species in the region of the $\alpha_1\beta_1$ contact is the presence of three buried weakly acidic groups—two cysteines and one tyrosine—which will ionize above pH 12 in Hb-A, and which are replaced by neutral or more weakly acidic groups in bovine haemoglobin. The ionisation and hydration of these groups perturbs the tertiary structure and therefore shifts the equilibrium towards the unfolded state and favours dissociation across the $\alpha_1\beta_1$ interface. Comparing Hb-A and Hb-F, the non- α or γ chains of the latter species provide one less cysteine and one less tyrosine in the same locations. These are two of the three changes, relative to Hb-A, found in bovine β chains. It is in fact possible to list several haemoglobin species in order of increasing resistance to alkaline denaturation, and to show that this order follows the progressive substitution of β -130 tyrosine, β -112 cysteine and, finally, α -104 cysteine by neutral or more weakly acidic residues.

Thus both qualitatively and semi-quantitatively, Perutz's suggestion that the ionisation of buried weakly acidic side chains determines the relative susceptibilities of different haemoglobin species to alkaline denaturation seems to be well founded. Certainly electrostatic effects involving exposed ionising groups are likely to be involved in the denaturation of monomers, but the ionisation of buried weakly acidic groups, notably cysteines, in the dimer interface region seems to be the crucial factor in promoting monomer formation at high pH and initiating the sequence of events leading to denaturation.

From a Correspondent

Herpes not so simplex

THE most recent discovery of Sabin and his colleague Tarro may open up at least two interesting avenues of research, both of which will be pursued at the University of Chicago, but each of which lead in an entirely different direction. Sabin announced early last year the finding, now published (Sabin and Tarro, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3225; 1973), that the serum of patients with certain types of tumour contains antibody to a substance produced by cells infected with herpes simplex virus (HSV). The discovery, which swells the growing body of evidence for the involvement of herpes viruses in human cancer, (see, for example, *Oncogenesis and Herpesvirus*, edit. by P. M. Biggs, G. de Thé and L. N. Payne; International Agency for Research on Cancer, 1972) is of immediate practical interest because the presence of antibody is associated with metastasis and may thus constitute a diagnostic tool for monitoring the progress of malignant disease. But its more far reaching implications depend on the possibility that the antigen is specified by a fragment of the HSV genome expressed in the tumour cells. From the practical point of view, this

would establish the rationale for developing the anti-viral vaccine which Sabin has envisaged as a prophylactic against human cancer. From the point of view of basic research, the antigen seems at last a palpable tool for probing the mechanism of carcinogenic transformation of human cells.

But although Sabin and Tarro refer to it as a nonviral antigen of HSV, no definitive evidence yet exists that it is specified by viral DNA. The evidence linking the tumour antigen with HSV is strong but circumstantial: all tumour types which have so far produced positive serum reactions in complement fixation tests occupy sites which would be accessible to HSV in a typical infection of the mouth, by HSV type I, or the genitals, by HSV type II.

Lac repressor and operator sequenced

How does repressor select one out of several million nucleotide sequences and bind to it to prevent the expression of the operon genes? In order to understand repression one must understand this and the stereochemistry underlying it. Two signal advances to this end have been reported by Gilbert and Maxam and by Beyreuther, Adler, Geisler and Klemm in the current issue of the *Proceedings of the National Academy of Sciences* (**70**, 3576 and 3581; 1973). Briefly Gilbert and Maxam have sequenced the operator, a stretch of DNA which binds the repressor of the *lac* operon in *Escherichia coli*; Beyreuther *et al.* have sequenced the repressor itself.

Gilbert and Maxam made use of the fact that the purified repressor will bind to cellulose nitrate those fragments of sonicated DNA which carry the operator. After binding in this manner the washed fragments were then eluted with a synthetic inducer. The repressor was found to bind to these purified fragments with a half life of 15 min so a quick treatment with DNase could be used to digest away all sequences except those protected by the repressor. The result was a double stranded DNA with a T_m of 67° C in SSC and containing twenty-seven base pairs as revealed by a calibrated polyacrylamide gel.

The sequence of this DNA was ascertained by copying one or other strands using RNA polymerase and complementary oligonucleotides of different length of sequence to effect initiation at various points. Fingerprinting of the transcription products yielded the complete sequence.

A feature of some interest in the final result was an ACAATT sequence near the 3' end of each strand (with a complementary sequence near both 5' ends), which gave the operator an element of symmetry. The two regions giving this symmetry are separated by nine base pairs and four of these show the same pattern of symmetry; the two regions are about one helix turn apart and thus could be approached from one side by a repressor showing a two-fold symmetry.

The repressor itself turns out to consist of four identical subunits each of 347 residues. No similarities with the sequence of histones or the known part of the β galactosidase were detected. Exactly how the two bind together must await further investigation, but Gilbert and Maxam suggest possibilities like opening up the DNA to see the bases directly or feeling out the edges of the bases in the grooves.

From a Correspondent

Sabin obtains the antigen from massively infected cultures of rabbit cells, in which it makes a brief appearance 8 h after infection, or guinea pig cells, in which it is synthesized about 3 h after infection (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1032; 1973). The designation non-virion rests on the persistence of reactivity in serum which has been absorbed with the structural components of HSV. The instability revealed by Sabin's tests implies either that it is rapidly degraded or that it is an aggregate of molecules the antigenicity of which is different from those of its components. It is reasonably clear that it arises as a consequence of HSV infection; but it is not at all clear that it does not represent a newly synthesised or modified preexisting host antigen. Similarly, the antigen which induces antibody in cancer patients may result, by chance, from similar but independent modification of human cell components.

The true identity of the antigen is the point which the laboratory of Roizman, at the University of Chicago, is well equipped to clear up. This group has been engaged for the past five years in a comprehensive analysis of the polypeptides specified by HSV and their synthesis in infected cells. They are also interested, though from a rather more academic point of view than Sabin's, in the involvement of HSV in the aetiology of cancer. At any rate, some of their resources have now been recruited to the pursuit of the putative nonvirion antigen, with the use of Sabin's sera to precipitate the polypeptide(s) involved. If they succeed, Sabin will have established the incrimination of HSV for all practical purposes; although, of course, the practical problems do not end with the identification of the causative agent.

The problems which would be encountered in an attempt to reproduce with HSV Sabin's achievement with poliomyelitis are legion. The potential dangers of a vaccine containing a virus which can remain latent in human cells for years and which, even when killed, can transform cells (Duff and Rapp, *Nature*, **233**, 48; 1971) need no emphasis. Even supposing it proved possible to isolate a mutant which neither remained latent nor transformed cells *in vitro*, on which to base a killed vaccine from which the viral DNA was removed, it seems likely that safety criteria for testing on human populations would be very hard to establish. Besides which, the vaccine might prove relatively ineffective in preventing infection, which can occur even in the presence of circulating antibody.

From Roizman's point of view, on the other hand, an established viral product expressed in tumour cells would provide the link he needs between two parallel lines of work in his laboratory.

One concerns polypeptide synthesis and its regulation in infected cells, which involves separation of the viral polypeptides on high resolution gels at various stages of infection. This has so far revealed forty-seven viral polypeptides, between sixteen and twenty-six of which are non-structural and therefore possible candidates for Sabin's and Tarro's non-virion antigen. They can be further classified into five different groups according to their rates of synthesis at different stages of infection (Honest and Roizman, *J. Virol.*, **12**, 1347; 1973). The other line of work concerns the fragments of the HSV II genome detected by N. Frenkel, with Roizman, in the cells of tumours of the uterine cervix. Frenkel is following up the discovery with studies of DNA hybridisation of HSV fragments from different cervical tumours, in an attempt to isolate the sequence common to all of them. Sabin's and Tarro's non-virion antigen could represent a product of the common genome fragment to which a specific function might possibly be assigned on the basis of the continuing investigation of the synthetic programme in HSV infected cells.

It would be a mistake to suppose that because the unstable nonvirion antigen is the only possible product of HSV so far detectable in tumour cells, it must necessarily be the functionally crucial product. But as the only one on hand, it is inevitably suspect as the transforming agent itself. In this case, according to his current hypothesis on the activities of HSV, Roizman would expect to find, not that the antigen would trigger mitosis, but that once mitosis had been initiated, it would keep the cell in the mitotic cycle. He has suggested that HSV remains latent only in differentiated cells which do not normally divide: on mitosis, the cell produces a factor which is necessary for the initiation of viral transcription. When the entire genome is present in the cell, expression results in the production of infectious virus and the destruction of the cell. But if, as in the case of tumour cells, only a portion of the genome is present, expression results in transformation of the cell.

To establish the role, if any, of the unstable antigen in such a mechanism, substantial advances in the fundamental understanding of HSV function would be necessary. Assigning regulatory functions to viral products will involve, among other things, the use of numerous mutant strains of HSV. Here, once again, Sabin and Roizman have an interest in common. Sabin needs HSV mutants from which to select one suitable for development as a vaccine. Roizman needs them to tie viral function to viral product. Perhaps coordinators of cancer research should give

up urging pure and applied researchers to seek conceptual common ground in favour of getting them together on purely technical requirements.

From our Special Correspondent

Dark imaginings

from our Experimental Psychology Correspondent

ONE of the reasons for the current interest in visual imagery is the question why a picture is worth a thousand words. Certainly this issue is very much the core of Allan Paivio's influential book *Imagery and Verbal Processes* (1971) and is, I suspect, also behind the recent paper of Neisser and Kerr of Cornell University on spatial and mnemonic properties of visual images (*Cognit. Psychol.*, **5**, 138; 1973). In two experiments Neisser and Kerr succeed in giving further confirmation of one answer to the question: visual images do provide a powerful aid to memory. But they also invite a series of related conclusions about the nature of spatial thinking and of perception.

It is worth mentioning that psychological interest in visual imagery as a mnemonic device dates back at least to classical Greece. The classical method, which is described in Frances Yates's admirable book *The Art of Memory*, is based on the proposition that sequences of concepts, such as one might want to use in giving a speech, are best committed to memory by imagining oneself walking round a familiar building, and creating in the successive places that one passes, visual images of the things one wants to mention.

There were within the theory all sorts of instructions about how to create memorable images: they should be concrete, striking, with clearly defined visual properties such as being beautiful or grotesque. There were also rules about the best kind of building in which they were to be put. It should be thoroughly familiar, but not crowded with people. It should not be of an architecturally repetitive style, but it should have discriminable locations, such as niches or corners of rooms, which should not be dark or in shadow.

More recently, and now cast into the arguably more rigorous experimental paradigm, these rules of visual mnemonics have been both re-established and extended. Thus it has been shown that just as in the ancient art a well-known building created an associative structure, so too, if one wishes to remember an association between two words, say, dog and bicycle when learning a list of such word pairs, the most efficient strategy is to visualise a scene in which some interaction between the

dog and the bicycle occurs: say, an image of a dog riding a bicycle, or being run over by one. This produces better recall of the second word bicycle when cued with the first, than does the creation of separate images of dog and bicycle or than an attempt simply to remember the words.

This, then, is the background of Neisser and Kerr's experiments. Subjects were given a series of twenty-four descriptive sentences, asked to form visual images of what was described in each, and to rate each image for vividness. For eight of the sentences the scene could be pictured directly, for example, "A harp on the top of the torch of the Statue of Liberty". For eight there was a connection between the two objects, but one that could not be captured in a single picture because of concealment; so the corresponding sentence would be "A harp inside the torch of the Statue of Liberty". For the remaining eight there was no direct connection, for example, "Out of one window I can see a harp and out of the other I can see the Statue of Liberty".

The second experiment of Neisser and Kerr provided some obvious controls for complexity and length of the sentences, and added a condition in which there was minimal luminous contrast between the two objects to be identified, for example, a piece of coal in a dark windowless cellar. The subjects were not warned in either experiment that they would have a recall test, but Neisser and Kerr found that when cued with the context given in each sentence (for example, the torch of the Statue of Liberty which for any one subject would occur in only one of their twenty-four sentences) subjects were able to recall the other object mentioned in the sentence equally well whether the image were pictorially possible, or when the object was concealed, or when there was low visual contrast between the object and its context. For disconnected images, however, recall was substantially worse, and there was furthermore no correlation between either time taken to form the image or its vividness when the sentences were first presented, and its recall subsequently.

Neisser and Kerr conclude that the mnemonic power of images depends upon their being representations of spatial relationships, not upon their possibilities for comprising a good 'picture' as such. The lack of decrement in the poor visual contrast images, for example, coal in a cellar, seems to be one point of contradiction with the rules of the ancient art of memory, which proposed that the loci in which images were to be placed must be well lit.

Just as interesting as the use of visualisation in memory are the implications that this kind of investigation

has for perception and thinking. Neisser and Kerr do not fail to relate their findings, that visual images are three-dimensional representations of layout rather than two-dimensional pictures of scenes, to an understanding of visual perception. When one sees, one does not experience retinal pictures as such, but the spatial layout of the environment. What they might also have pointed out, but did not, is that human beings seem to possess specialised and powerful mechanisms for reasoning about spatial relationships. They form the basis of visual perception and Helmholtz referred to them as processes of "unconscious inference". In the Renaissance the ancient art of memory became transformed from a process of simply recording static images to a method that actually allowed the powerful mechanisms of spatial conceptualisation and inference to help one to think. Perhaps soon one may also look forward to some experimental exploration of this aspect of the uses of imagery.

Interaction between pineal and pituitary glands

from our Steroid Biochemistry Correspondent

THE pineal gland has been studied intensively in recent years and the view is growing that this gland can affect several endocrine functions in the body. It is known to inhibit reproductive functions in mammals. Although the mechanisms by which the pineal acts are not fully understood, it is thought that light, and possibly other factors such as cold, stress or other hormones, act on it to decrease the synthesis of melatonin and/or serotonin by inhibiting specific enzymes needed for the formation of these compounds. Production of melatonin in the pineal is stimulated by darkness and inhibited by light. Several questions about the synthesis, secretion and transportation of melatonin and its effect on different endocrine glands, however, remain unresolved. Do these compounds act peripherally at specific sites or is their main site of action within the brain affecting trophic hormone release? Are they secreted into both the blood stream and cerebrospinal fluid?

Further information relating to some of these questions has now been provided by Seibel and Schweisthal (*Acta endocr., Copnh.*, **74**, 434; 1973), who used the golden hamster to study the relation between the pineal and other endocrine glands. Although melatonin or serotonin have not yet been isolated

from the pineal gland of the hamster, this animal has the advantage in studies of the effects of changes in environmental lighting and pinealectomy, that the changes in the gonads are much greater than in the albino rat.

The mean weights of testes and seminal vesicles of hamsters blinded by bilateral enucleation were significantly lower than those of control, pinealectomised or blinded and pinealectomised animals, but could be increased by melatonin, whereas after hemi-castration, pinealectomy with hemi-castration or with hemi-castration and blinding there was a significant increase in the mean weight of the testes. Mean testicular weight was not altered by bilateral enucleation combined with hemi-castration suggesting an interaction between the pineal antigonadotrophic substances and the pituitary gonadotrophins, but was reduced on treatment with melatonin and serotonin.

No significant changes in the weights of the adrenal or pituitary were found in any experimental conditions except in the group which underwent all three operative procedures and were also treated with melatonin. These results differ from those obtained in the rat where regression of the adrenals occurs after blinding. The increased uptake of iodine-131 observed in blinded animals could be reduced by melatonin treatment. After parabiotic union in males, whether one or both animals were blinded, there was a significant decrease in testicular weight indicating that the pineal antigonadotrophic substance was carried through the blood.

These investigations show an interaction between the secretions of the pineal and those of the pituitary. Melatonin is known to depress secretion of luteinizing hormone most probably by an effect on the hypothalamus. So does serotonin which will also depress secretion of follicle stimulating hormone and the relations between these two biogenic amines needs clarification.

Genetic manipulation

from a Correspondent

GENETIC manipulation by the biologist has, in the past few years, become an area of great controversy and increased research activity. This has led, in some cases, to workers trying to jump on the genetic engineering bandwagon when their results are only minimally applicable to the field.

Levy, Snyderman, Ladda and Lieberman (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 3125; 1973) attempt to show that a genetic deficiency can be temporarily

repaired after the inoculation of Sendai virus fused cells *in vivo*. The recipient animals are mice which are deficient in the ability to produce the fifth component (C5) of complement. The inoculated cells are splenic macrophages fused to either kidney cells from a closely related mouse strain (C5 positive) or chicken erythrocytes. Splenic macrophages are thought to be at least one of the cells involved in the production of complement whereas neither mouse kidney cells nor chicken erythrocytes are involved in C5 formation.

Sera taken from the inoculated animals were shown to contain antigenically-related mouse C5 as well as both haemolytic and biological complement activity. The C5 activity reached its peak at 3-6 d after inoculation and then declined reaching background levels after 21 d. The decline in C5 activity may be due to the production of anti-C5 antibody by the recipient animals. Possibly the simplest explanation is that the environment for the production of C5 is defective in the mouse splenic macrophages. This defective environment may be complemented by a non-defective cell, even a chicken erythrocyte, to cause the production of mouse C5.

These results are no doubt interesting but do not seem to warrant the authors' speculation that their experiments may provide a prototype for genetic repair by hybrid cells in humans. Although the authors continually talk about hybrid cells no evidence is shown that the fused cells indeed contain hybrid cells capable of division. Most cell biologists would refer to them as a heterokaryon-containing population. In any case fused cells are not essential for the result. Similar results were also obtained by Levy *et al.* by inoculating unfused non-defective splenic macrophages. As with the fused cells the repair with these macrophages is also only temporary. Even if the real and major problem of preventing the rejection of transplanted foreign cells could be overcome, viable functional cells (hybrid or not) which are capable of populating the appropriate areas within the recipient animals would be needed. Finally one should take into account the possible dangers to the host in such experiments. Fusion of cells can not only induce dormant viruses but can also result in differences in growth control and antigenic character in the hybrid cells produced.

A report more applicable to genetic manipulation is presented by Cohen, Chang, Boyer and Helling (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 3240; 1973). Using the restriction enzyme *Eco* RI these workers have been able to construct new biologically-active plasmid DNA molecules which are capable of replication. *Eco* RI cleaves double

stranded DNA at a unique site producing short, overlapping single stranded ends. The overlapping sequences are self complementary and will hydrogen bond to each other allowing the nicked molecules to be resealed by DNA ligase *in vitro* or *in vivo*. By these means fragments from the same or different sources can be joined together.

Cohen *et al.* utilized bacterial plasmids which contain a number of drug resistance markers. Transformation was performed with the *Eco* RI endonuclease cleavage products of a plasmid DNA containing twelve cleavage sites. By selecting for resistance to only some of the markers Cohen *et al.* were able to isolate a clone of bacterial cells which contained a plasmid with only three *Eco* RI fragments. By mixing the *Eco* RI fragments of two different plasmids and using similar procedures hybrid plasmids were obtained. In all cases it was shown that the new plasmids were most likely derived from the joining together of *Eco* RI fragments. By identification of the fragments present and of the drug sensitivity of the plasmid an assignment of drug resistance to particular fragments was then made.

Every combination of restriction enzyme fragment hybrids can probably not be isolated. In order to be able to isolate the hybrid molecule a functional system for replication and transcription must be present. Since fragmentation and subsequent hybrid molecule formation depends on DNA sequences and not on the origin of the DNA, this method may well be used as the authors point out for inserting and growing up DNA fragments from a variety of sources. If DNA from higher organisms can be tolerated in bacterial cells, one will no doubt soon see the isolation of many genes. This procedure is not necessarily restricted to the use of *Eco* RI as there are a number of other restriction enzymes which produce overlapping single stranded ends and cleave at other unique sequences in the DNA.

Indirect effects of pesticides

from a Correspondent

WAYS in which pesticides may affect the host plant, besides controlling pests and diseases, were considered by the Pesticides Group, Society of Chemical Industry, on November 6 under the chairmanship of R. A. E. Galley. Damage to a crop can take many forms and in some the symptoms are obvious. Effects on yield, though more important, are less easy to detect. Several speakers stressed the difficulty in demonstrating statistical

significance even for a 10% change. They found that attempts to relate yield to dose of pesticide often led to unexpected results.

Petroleum oil emulsions, used to control red spider mite in glasshouses, may reduce cucumber yields by about 10% when sprayed every 10 days throughout the 8-month crop. N. W. Hussey (Glasshouse Crops Research Institute) described the symptoms produced by various oil fractions on young tomato plants. Single sprays applied undiluted at forty times the normal quantity showed that even a low aromatic content can cause damage, especially with oils of low viscosity. Removal of the aromatic constituents by percolation through silica gel largely prevents damage to both cucumbers and tomatoes. Trials are continuing on cucumbers sprayed with dearomatised oils at normal rates through the season.

D. J. Butt (East Malling Research Station) discussed hazards to shoots, blossoms and fruits arising from fungicide sprays used on apple trees. Fungicides interact with cultivars, time of season and weather, and must be selected and timed so that disease control is achieved with minimal phytotoxicity. The flowering period is vulnerable and, although mildew control is necessary at this time, dinocap and especially binapacryl can reduce fruit set. On occasions benomyl has increased fruit set. Crop quality can be reduced, thus binapacryl applied after June may cause spotting. The most insidious type of damage is russetting, and fruit skins are sensitive to certain fungicides in May and June. The systemic fungicides available tend to induce russet as does dodine when used near 0° C. Some fungicides ameliorate russet, and captan and bis(dimethylthio-carbamoylthio)methylarsine have been successful as palliatives (*Ann. appl. Biol.*, **75**, 217; 1973).

Factors contributing to the phytotoxicity of cereal seed dressings were considered by K. A. Jeffs (Rothamsted Experimental Station). Wheat plants from seed treated with gamma-BHC are less damaged in peaty than in sandy soil, but chlorfenvinphos has caused damage in peaty soil. Adhesives to stick powder dressings to seeds may enhance phytotoxicity, as may liquid formulations. Experiments with gamma-BHC liquid dressing showed that only seeds treated near the embryo were damaged and amounts of insecticide translocated into the plant from treated seeds were variable and small.

F. Baranyovits (Plant Protection Ltd) illustrated the safety of two insecticides to plants. Pirimicarb is systemic when applied to soil, being absorbed by the roots and transported through the xylem. When high doses are applied it ac-

accumulates at the lower leaf margins of chrysanthemums causing necrosis. Its distribution has been confirmed by autoradiography with ^{14}C -pirimicarb-treated plants. Pirimicarb smokes are safe at the recommended rate but an eight-fold increase produced varied damage. Thus with carnations the growing tips were scorched, only the older leaves of cucumbers were damaged, cyclamen were unharmed, and primula petals unaffected whereas the leaves were damaged. An experimental pirimiphos-methyl formulation produced localised scorch on citrus fruit when spray droplets dried concentrating the insecticide. No damage occurred with a formulation that dried more uniformly.

Flies in mushroom houses are controlled by mixing insecticides with the casing layer or with the compost though yield reductions have been suspected. I. J. Wyatt (Glasshouse Crops Research Institute) described trials in which diazinon and chlorfenvinphos (from 10 to 2,000 mg kg⁻¹ casing) reduced mushroom numbers in the early flushes and increased them in the later. When numbers were decreased, however, the mushrooms were larger, resulting in increased yields with low rates of insecticide. The compounds were more persistent in the compost and tended to reduce yields in all flushes. A simple computer-fitted model described the behaviour. G. A. Wheatley (National Vegetable Research Station) also described unexpected effects produced by insecticides in trials. Chlorfenvinphos gave good control of cabbage root fly damage on mini-cauliflowers but the yield was less than with diazinon, which gave poor control. On conventional cauliflowers, curd diameter increased progressively with increasing diazinon dose, but not with chlorfenvinphos or fonofos although they gave better protection against cabbage root fly larvae. Phorate and *S*-tert-butyl *OO*-diethylphosphorodithioate controlled carrot fly and affected plant survival. Although total yield did not change much, the size of the carrots was affected and hence their suitability for specific markets. Different ways of using chlorfenvinphos altered sizes of Brussels sprouts with similar implications.

E. Griffiths (University College of Wales, Aberystwyth) reviewed the effects of fungicides on leaf rust and coffee berry disease affecting coffee in East Africa. One spray of Bordeaux mixture doubled yields even in the apparent absence of disease. It became an annual treatment known as 'tonic copper spraying', though other fungicides produced the same effect and both diseases can be aggravated. Without further control measures rust may later develop more quickly in the thickly foliated trees that result from 'tonic spraying'. Coffee berry disease

infection can come from berries or the fungus living saprophytically in the bark of maturing shoots. Fresh spores from bark sprayed 14 months earlier are more infective than those from untreated trees. Do the fungicides affect the tree or other microorganisms?

Introducing a lively discussion, Q. A. Geering (Fisons Ltd, Agrochemical Division) stressed that many of the effects described had been obtained with higher doses of pesticides than used commercially.

Insulator layer formed in silicon

from our
Solid State Physics Correspondent

THE frequency of publication on ion implantation indicates that this technique may be catching on as a technological tool (see, for example, the recent book *Ion Implantation* by G. Dearneale, J. H. Freeman, R. S. Nelson and J. Stephen (North-Holland, Amsterdam, 1973)). In the research journals and at conferences, every week seems to bring a new idea for making use of ion implants in microelectronics. A beam of ions projected at kilovolt energies into the surface of an electronic material forms a burned layer of implant atoms, in a matrix of atoms of the host material. The properties of that layer are, of course, different from the original material. For semiconductors it has been realised for some time that by varying the ion used, its energy and the subsequent heat treatments, one can form either a layer which is more strongly conductive than the original semiconductor or a glassy layer which is still a semiconductor but much less conductive. It has only been clear since about 1970, however, that, by implanting a high concentration of a reactive ion, a layer of insulating compound can also be formed.

Dexter and Watelski of Texas Instruments, Dallas, and Picaux of Sandia Laboratories, Albuquerque, (*Appl. Phys. Lett.*, **23**, 455; 1973) have now reported a particularly useful application of that finding. They have used bombardment wafers of silicon with nitrogen ions to form a buried silicon nitride layer and then, by etching grooves, they have produced slabs or 'mesas' of silicon which are isolated from the body of the silicon wafer. 'Dielectrically isolated' devices can then be formed in these slabs—a useful form of device in the preparation of fast interference-free integrated circuits. The surprising and novel feature of the implantation method was that, even with the high ion dose used (more than 10^{17} ions cm⁻²) the silicon

left above the buried layer was free of defects after heat treatment at 1,200° C. Proton channeling showed a near-perfect lattice and the silicon would support the epitaxial growth of a similarly defect-free crystalline silicon film of thickness 0.2 μm . The important point about the film quality was its superiority in comparison with a competing process, namely the epitaxial growth of silicon films on sapphire. The latter process has been used to manufacture integrated circuits but suffers from some drawbacks such as inhomogeneity of impurities and defects in the silicon and expensiveness and difficulty in working the sapphire.

The buried 'nitride' films had a breakdown strength which approached that of pure silicon nitride, breaking down at a field of 5×10^5 V cm⁻¹, although, theoretically, there is not enough nitrogen present to form the compound Si₃N₄. It seems that it will be possible to form diffused transistors and diodes in this silicon layer of 0.2 μm and interconnect them into an integrated circuit. This method could prove cheaper, simpler and hence more reliable than the present methods for dielectric isolation which, with the exception of the silicon-on-sapphire technique, involve the difficult stage of lapping off the body of the silicon wafer after mesas, formed by etching, have been somehow secured in a solid dielectric matrix.

A warning against overoptimism concerning this method should, however, be inferred from the almost simultaneous publication of an article describing unexpected migration of defects beyond the range of implanted ions themselves. Clearly, high dose implantation and the required high temperature anneals which follow, place a large stress on the electronic materials involved which may lead to new, unexpected and frustrating forms of damage effect.

Superfluidity in thin helium films

from our
Condensed Matter Correspondent

THE superfluid content of very thin adsorbed helium films has been measured directly, using a quartz crystal as a microbalance, by Chester and Yang of the University of California, Los Angeles (*Phys. Rev. Lett.*, **31**, 1377; 1973).

Bulk liquid ⁴He below T_λ, its superfluid transition temperature, behaves in many ways as though it were a mixture of two entirely different but completely interpenetrating fluids a so-called normal fluid component which, with finite viscosity and entropy, is much like any other liquid; and a superfluid component

which, having zero viscosity and entropy, is responsible for many of the remarkable phenomena associated with liquid helium. The proportion of superfluid varies from unity at 0 K to zero at T_λ .

Much interest has been attached to the behaviour of helium in confined geometries and, in particular, to the properties of adsorbed helium films held on glass or metal surfaces by the Van der Waals force. It is well known that superflow occurs readily in the relatively thick (100 atomic layers) films which occur in equilibrium under the saturated vapour pressure. An intriguing question arises, however, as to just how thin a helium film can be made before its superfluidity disappears and this is one of the problems to which Chester and Yang have addressed themselves.

Their apparatus consisted of a quartz crystal, 0.07 mm thick and 6.3 mm in diameter, vibrating in its thickness-shear mode such that the oscillations of the surface were parallel to itself. In this situation the characteristic frequency of the crystal is very sensitive to the mass of any material deposited on its surface and the mass of an adsorbate can be determined accurately by measuring the resultant drop in frequency. In the case of a helium film, however, only the normal fluid component is able to follow the oscillations of the crystal. The superfluid component, because of its zero viscosity, is not coupled to transverse oscillations of the surface, remains motionless and therefore does not contribute at all to the loading effect of the helium film. The technique thus enabled direct measurements to be made of the mass of normal fluid in the film, under various conditions of temperature and film thickness.

The film thickness at any particular temperature T was controlled by adjusting the pressure P of the helium gas surrounding the crystal and with which the film was in equilibrium. The mass σ , which was loading unit area of the crystal at first increased with P , suddenly fell by a small factor and then increased more slowly as P continued to rise towards the saturated vapour pressure, P_0 . Chester and Yang interpret these results as indicating that at very small values of P (very thin films) the film displays no superfluidity; they attribute the abrupt drop in σ to the onset of superfluidity with an associated decrease in the effective mass loading the crystal; and they point out that the subsequent slower rise with P of σ_0 is to be expected, assuming that only the additional normal fluid will load the crystal. This interpretation is consistent with their earlier paper (*Phys. Rev. Lett.*, **29**, 211; 1972) with Stephens of the California Institute of Technology in which they presented preliminary data and, in particular, reported that similar measurements for an adsorbed film of ^3He , which is not a

superfluid at these temperatures, yielded a monotonic dependence of σ , on P , from zero right up to P_0 , with no sign of the features attributed to superfluidity in the present case of the ^4He films.

To deduce the proportion of superfluid component from the experimental values of σ , it was, of course, necessary to know the total mass σ per unit area of the film for any given values of T and P . By replotting their values of σ , against $(P/P_0)^T$ as abscissa, the authors have succeeded in generating a universal curve relating σ to P and T , as required. Each experimental curve started out from the origin along the universal curve and then, at some particular value of $(P/P_0)^T$, depending on T , started to deviate because of the presence of superfluid. By measuring the magnitudes of these deviations they were able to determine the mass σ_s of superfluid component per unit area corresponding to any particular value of σ , for a variety of temperatures.

After making additional assumptions, consistent with a number of earlier experiments, that the first adsorbed layer is solid, and that the second and subsequent layers are liquid of the standard density, the authors conclude that superfluidity does not occur in a helium film until between two and three atomic layers of liquid, depending on the temperature, have been deposited.

X-ray 'star' in Cygnus Loop

RAPPAPORT *et al.* report that they have detected a central 'hot spot' in the Cygnus Loop. This source, detected at X-ray frequencies during a non-dimensional scan from an Aerobee rocket on March 30, 1973, may be the stellar remnant of the supernova explosion, *Astrophys. J. Lett.*, **186**, L115; 1973). If so, it represents only the third definite identification of such a stellar remnant.

In a sense, this discovery completes a set of such identifications, for the central star in the Crab Nebula was first found with optical telescopes, and the equivalent source in Vela was first detected at radio frequencies, as the Vela pulsar. Both these other two objects, of course, have X-ray counterparts.

The best position quoted for the Cygnus object is $\alpha(1950) = 20^{\text{h}} 49^{\text{m}} 40^{\text{s}}$; $\delta(1950) = 30^\circ 43' 5''$. But the error box only marginally overlaps with that of the 'hot spot' reported by Stevens and Garmire (*Astrophys. J. Lett.*, **180**, L19; 1973), which lies distinctly off centre in the Cygnus Loop. The available observations are fitted by a black-

body spectrum with temperature $1.9 \pm 0.6 \times 10^6$ K, and X-ray luminosity of 8×10^{34} erg, assuming a distance of 770 pc. It is interesting, as Rappaport *et al.* point out, that these parameters correspond to an object about 30 km across, in the right area for a neutron star. Also, there is some evidence of periodic variations of the source with a frequency of 16 Hz, again about right for a spinning neutron star.

Swings, roundabouts and Sco X-1

from a Correspondent

THAT there is a source mechanism which can give rise to periodic pulsed radiation in close, interacting binary stars is rapidly becoming an established observational fact. A wide range of apparently differing systems which exhibit a variety of pulsing behaviour are being found.

The regular periodic pulses observed in the X-ray systems Cen X-3 and Her X-1, superficially similar to the on-off, on-off radio pulses of pulsars, are now well known. The periods, of the order of a few seconds, are so short that it has become widely accepted that a highly compact neutron star must be involved (although while dwarf models cannot yet be ruled out—see *Nature*, this issue, page 333). The widely accepted explanation of these stable pulses is that they are produced by the infall of material, flowing from the normal stellar component, and accreting preferentially along magnetic field lines onto a neutron star companion. If the material is funnelled in this way onto the magnetic poles and the neutron star rotates with the required period of a few seconds, the observed X-ray pulses are, at least qualitatively, easy to explain.

In the case of the strongest compact X-ray source, Sco X-1, no such stable pulsing behaviour has been found, suggesting the absence of a strong magnetic field. But several claims have been made that weak periodicities of a transient nature can be observed, in particular in the optical region. These are the subject of a recent paper (Frohlich, *Mon. Not. R. astr. Soc.*, **165**, 313; 1973) which may help clarify the issue.

The optical counterpart of Sco X-1 is known to be a variable star exhibiting very rapid light fluctuations, and additional larger scale flaring on a timescale of a few hours. The apparently erratic optical fluctuations have been analysed by various investigators using power spectra and the so-called periodogram techniques in a search for evidence of

underlying periodic behaviour, with somewhat discrepant results. Of a total until now of seven investigations, only two teams found evidence of periodic behaviour; one group reported a transient pulse with a period of 20 s in both the optical and X-ray region; the other group suggested the detection of pulses in the optical with periods of 117 and 182 s coexisting.

The existence of pulses in Sco X-1 has therefore been a matter of some controversy. But the latest observations of Frohlich cannot be lightly dismissed. On four successive days in June 1972, evidence was found for the presence of low amplitude optical pulses with a period of 121 s. This agrees quite well with at least one of the periods found by an earlier group (P. A. Peldman *et al.*, *Nature*, **225**, 1123; 1970). A further fact, which if confirmed might help in the interpretation of these pulses, was a correlation between the brightness of the source and the period observed. On one night the overall output declined by a factor two in half an hour. This was associated with a decrease in the pulse period from 121 s to 118 s. Although Frohlich does not speculate as to the origin of these weak, transient pulses or this apparent brightness period correlation, a whole range of questions and possibilities immediately come to mind.

The sort of behaviour reported in Sco X-1 is remarkably similar to that of another class of binary system, the so-called dwarf novae. These show similar weak transient pulses which change in their period as the brightness changes. The dwarf novae are distinguished as a class by their explosive outbursts, brightening suddenly by several orders of magnitude and then more slowly declining back to a quiescent state. The outbursts recur at irregular, but approximately monthly intervals. The origin of these outbursts and of the underlying transient pulses remains a question of debate. But we might hope that the observation of somewhat similar pulse behaviour in Sco X-1 may help to elucidate the phenomena occurring in both types of system.

Two main lines of approach to account for transient pulses have so far been suggested: either oscillations, similar in some ways to the oscillations of the well known Cepheid pulsating stars; or rotation, as in the pulsars and stable pulsing X-ray sources mentioned earlier. Thus the choice seems to lie between what might be called swings and roundabouts. Both have been advanced to explain the pulses in dwarf novae. Put simply the swings model accounts for the pulses as being due to oscillations of the envelope of the accreting compact component, the pulses being excited by the nova outbursts themselves which are

ascribed to phases of unstable nuclear burning on the accreting white dwarf. The roundabout model ascribes the outbursts to the sudden dumping of matter onto the dwarf as its companion undergoes phases of unstable mass outflow. It is well known that as a consequence of its angular momentum this accreting material must spiral slowly in onto the white dwarf rather than falling straight onto the surface. It is suggested that it is just irregularities in this inward spiralling disk that gives rise to the pulse phenomenon.

It will be interesting to see how the roundabouts and swings fare in explaining in their rival fashions the behaviour of Sco X-1. Perhaps one shall then be able to get an answer to the truth or falsity of that old adage about gaining on swings and losing on roundabouts.

Radiation of life in the Precambrian

from our Palaeontology Correspondent

THE cause of the sudden explosive radiation of multicellular organisms near the beginning of the Cambrian period, after a long history of slow unicellular evolution, is widely regarded as the foremost unsolved problem in palaeontology. In recent years two rather different approaches have been adopted in an attempt to solve the problem: the first emphasised external physical controls such as the changing level of atmospheric oxygen, whereas the second concentrated on biological aspects, such as the advent of eukaryotic cell organisation, sexuality or the formation of preservable skeletons. Neither approach, however, has been generally accepted.

In an important review, Schopf *et al.* (*J. Paleont.*, **47**, 1; 1973) point out that the evidence of Precambrian fossils suggests that eukaryotic organisms arose more than 1,300 million years ago. The advent of sexuality should greatly have increased genetic variability and hence rates of evolutionary diversification, but the late Precambrian floras seem to be very impoverished in comparison with their living equivalents. The authors suggest that the long time gap, of 250–300 million years, between the origins of sexuality and multicellular body organisation might have been a consequence of the gradual nature of the trend from a haploid to a diploid-dominated life cycle. This explanation does not seem very convincing, because an important evolutionary innovation such as diploid cell division should have risen to dominance very quickly. Another explanation of the rapid radiation of metaphtes and

metazoans has been put forward by Stanley (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1486; 1973), who applies a newly established 'cropping' principle to the problem.

Ecologists have acquired a substantial body of evidence that the addition of a trophic level to a given food web tends to promote increased diversity at the next lower trophic level. Thus the removal of the chief natural carnivore from a community can lead to a drastic reduction in herbivore diversity as one or a few superior competitors among the latter achieve dominance. According to Stanley, the Precambrian trend from simple autotrophic producer to producer-herbivore and producer-herbivore-carnivore communities can be viewed as a long-term natural cropping experiment comparable to those performed by ecologists.

Initially an all-producer Precambrian organic world of bacteria and blue-green algae was characterised by low diversity and low rates of speciation. The system was self-limiting insofar as the changes needed to produce unicellular heterotrophs and metazoans had to arise slowly because diversification was slow. When cell-eating heterotrophs first arose in the late Precambrian they may have fed on bacteria. The adaptive breakthrough to feeding on algae led to the addition of successive trophic levels to the ecosystem. In general, diversification at any trophic level tends to promote diversification of the subjacent level by cropping and diversification of the suprajacent level by providing more feeding options. Thus a self-propagating mutual feedback system can arise. Stanley sees the explosive evolution of multicellular organisms as an inevitable consequence of the advent of heterotrophy. Supporting evidence is sought from the record of ancient stromatolites, which are laminated sedimentary structures produced by blue-green algal mats that trap and bind sediments. Their rapid decline at the close of the Precambrian has been considered to be the result of destruction of algal mats by animal grazers and burrowers, so that subsequently blue-green algae have only flourished in restricted environments, hostile to other organisms, such as supratidal flats. This suggests that blue-green algae and other photosynthetic autotrophs saturated aquatic environments until very late in the Precambrian.

It is obviously too early to assess what chance Stanley's theory has of being more popular than its predecessors. It does seem, however, to have several definite strengths: it accounts for a wide variety of facts, it is basically simple and is purely biological, with no *ad hoc* invocation of external physical controls.

Mechanism of denaturation of haemoglobin by alkali

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Denaturation by alkali is activated by the ionisation of buried, weakly acidic side chains. Species variations are largely accounted for by replacements of one buried tyrosine and two cysteines.

Two effects may contribute to the denaturation of a protein by acid or alkali. First, the free energy of a spherical polyelectrolyte is proportional to the square of its net surface charge, so that its stability decreases on either side of the isoelectric point as either its cationic or its anionic groups are being discharged. Second, titration with acid or alkali may ionise weak acids or bases that lie buried in the non-polar interior. On becoming ionised, these groups attract hydration shells which are misfits in the native structure and therefore shift the equilibrium towards the unfolded form.

Mammalian haemoglobins have molecular weights of 64,500 and consist of four haems and two pairs of polypeptide chains known as α and β . The α chains each contain 141 amino acid residues and the β chains 146. Their secondary structures are made up of seven or eight helical and six non-helical segments (Fig. 1). Their tertiary structures are held together by secondary valency forces, mainly hydrophobic interactions. Side chains carrying ionised groups or amides are excluded from the interior of the subunits, but cysteines, serines, threonines and tyrosines occur both inside and outside. The four subunits are arranged tetrahedrally around a two-fold symmetry axis. In oxyhaemoglobin, which is the form under discussion here, significant contacts are made only between unlike subunits and these are restricted by symmetry to two kinds called $\alpha_1\beta_1$ and $\alpha_1\beta_2$. In both these contacts hydrophobic interactions predominate^{1,2}. Carbonmonoxy and cyanomethaemoglobin have the same structure as oxyhaemoglobin.

Human and Bovine Haemoglobin

X-ray analyses and amino acid sequence studies show that the molecular architecture of all mammalian haemoglobins must be closely similar, yet they vary greatly in their susceptibility to denaturation by alkali³. For instance, in 0.1 N NaOH at 20° C adult and foetal human CO-haemoglobin are denatured with half times of 20 s and 12 min respectively, while bovine haemoglobin is stable. It takes 0.5 N NaOH to denature it with a half time of 3 h⁴. All three haemoglobins dissociate from tetramers into dimers above pH 10.0 (refs 4, 5). This step is reversible and not associated with any denaturation⁵. It is due to splitting of the molecule at the $\alpha_1\beta_2$ contact⁶ where the amino acid sequence is invariant, so that no significant species variations in the dissociation constant arise. The resulting dimer has the structure shown in Fig. 1 (ref. 7); its two subunits are held together at the $\alpha_1\beta_1$ contact which is made up of thirty-four residues or 110 atoms, mostly belonging to helices G and H and to the GH corner¹. When the pH of human adult CO-haemoglobin is raised to 11.6, this dimer splits apart and denaturation sets in⁴. It has been suggested that

the splitting is likely to be the rate-limiting step⁸. When considering its cause, it is useful to keep in mind the experience gained from the abnormal human haemoglobins which has shown that any amino acid replacement which opens the non-polar interior to water is likely to be a source of instability, and that this is true especially of buried non-compensated charges⁹.

The amino acid replacements that distinguish the three proteins are known from sequence studies¹⁰⁻¹⁴. The adult human and bovine forms differ at fifteen positions in the α and eighteen in the β chains. One of these differences is a deletion of residue NA1 which lies in the internal cavity of the human tetramer, and twenty-six are found in positions which, in the tetramer, are either external or lie in the internal cavity. In the $\alpha\beta$ dimer this cavity is wide open, so that these latter positions also become external. Of the remaining six differences, three are due to exchanges of non-polar residues, two of them interior (EF2 and EF5 α) and one lying in a surface crevice (A11 β). Of the replacements listed so far, the only ones to be affected by raising the pH from 10 to 13 are the lysines and arginines whose cationic groups have pKs of 10.5 and 12.5 respectively¹⁵. But the instability arising from their discharge is unlikely to have a decisive effect on the susceptibility to alkali denaturation, because there is no net difference between the number of basic groups in the two proteins and because human haemoglobin is known to remain native even after acetylation of 80% of its amino groups¹⁶. Of the remaining three positions, G11 α and G14 β are both occupied by cysteines in human adult, but by serine and valine re-

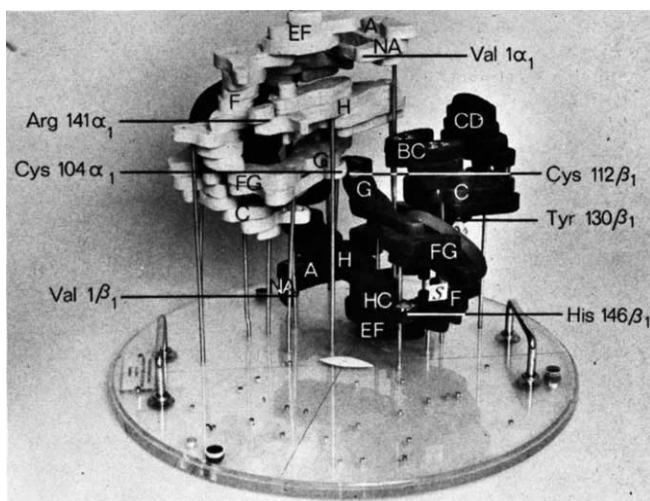


Fig. 1 Model of the $\alpha\beta$ dimer seen at low resolution. In going from the amino to the carboxyl end, helical segments are denoted A to H; non-helical segments at the amino end NA and at the carboxyl end HC. Non-helical segments between helical ones are denoted AB, BC and so on. Residues within each segment are numbered from the amino end (see Table 1). S marks cysteine 93 β . The white sign on the base marks the position of the two-fold axis in the tetramer.

TABLE 1 Susceptibility to alkali denaturation of various haemoglobins as determined by Singer test*

Species	Cys G11 (104) α	Cys G14 (112) β	Tyr H8 (130) β	% undenatured haemoglobin after 1 min at pH 12.7	$k^\dagger$
Human adult ¹⁰⁻¹²	+	+	+	~1	2.3×10^{-2}
Macaque (Rhesus) ²⁶	+	+	+	0 - 1.4	
Spider monkey ²⁴	+	+	+	0.5 - 0.7	
Capuchin monkey ²³	+	+	+	2.4 - 2.7	
Dog ²⁰	+	+	+	~3 $\ddagger$	2.8×10^{-4}
Rabbit ^{22,27}	+	—	+	?	
Slow loris ²⁵	+	—	+	53	
Human foetal ¹³	+	—	—	80	
Brown lemur ^{22§}	+	—	—	67	9.3×10^{-6}
Bovine ¹⁴	—	—	—	100	

* In the Singer test³⁶ 0.1 ml of a 10% solution of oxyhaemoglobin is added to 1.6 ml of N/12 NaOH (pH 12.7) at 20° C. After 1 min 3.4 ml of a solution made up to 800 ml 2 M (NH₄)₂SO₄ + 2 ml 10 N HCl are added and the mixture is filtered. The percentage of undenatured haemoglobin remaining in the filtrate is determined spectroscopically. The procedure was later modified by Beaven *et al.*,³⁷ using cyanomet- instead of oxyhaemoglobin but still measuring the percentage which remains undenatured after 1 min at pH 12.7 and 20° C.

$\dagger$ First order rate constant at pH 11.9 and 2° C (ref. 28).

$\ddagger$ Inferred from the rate of denaturation published by Huehns *et al.*¹⁹

$\S$ Based on peptide compositions only.

spectively in bovine, while H8 β is occupied by tyrosine in human adult and phenylalanine in bovine. All three positions are buried, G11 α and G14 β in the $\alpha_1\beta_1$ contact and H8 β between helices H and A. In water, the sulphhydryl groups of cysteine have a microscopic pK of 9.1–9.5 and the phenolic groups of tyrosines one of 9.5–10.0 (ref. 15). These groups might have their pK s raised if they lie buried, but they would certainly become ionised above pH 12. The pK of the hydroxyl group of N-acetylserine amide in water, on the other hand, is 13.6 (ref. 17), which would be raised further in serine G11 α in bovine haemoglobin, because it lies buried among the hydrophobic side chains. I conclude that the raising of the pH from 10.0 to 13.0 would lead to the ionisation of three buried and weakly acidic groups in human adult, but not in bovine, haemoglobin.

Human adult and foetal haemoglobins share the same α chains, but the β chains of the foetal form, usually referred to as γ , differ from the adult form in thirty-nine positions¹³. Twenty-nine of these are external in the $\alpha\beta$ dimer, seven involve replacements of one type of non-polar interior side chain by another, and one involves a replacement of an internal alanine in β by a threonine in γ . By the arguments set out above, none of these are likely to have a significant effect on the susceptibility to alkali denaturation. The remaining two replacements are of cysteine G14 in β by threonine in γ and of tyrosine H8 in β by tryptophan in γ . These are the same buried, weakly acidic groups which are replaced by neutral or more weakly acidic groups in the β chains of bovine haemoglobin. In summary, the differences in susceptibility to alkali denaturation are likely to arise because human adult haemoglobin has three buried side chains ionisable around pH 12.0, human foetal has only one, and bovine none (Table 1). The two buried cysteines lie at the $\alpha_1\beta_1$ contact; in the ionised form they would be hydrated and prevent the association of the subunits. Ionisation of the buried tyrosine would attract water into the non-polar interior of the β chain and tip the equilibrium towards the unfolded state.

Free γ chains associate to form tetramers and are more susceptible to alkali denaturation than native foetal haemoglobin¹⁸, probably because the dissociation constant of the equilibrium $\gamma_4 \rightleftharpoons 4\gamma$ is larger than that of the equilibrium $\alpha\gamma \rightleftharpoons \alpha + \gamma$.

Other species

Information about the rate of alkali denaturation is available for several other species though not always in comparable form. The half times of the denaturation reaction

of canine and adult human cyanomethaemoglobin at pH 12.7 and 20° C are similar: 20–25 s as compared to 11 s¹⁹. This is consistent with the presence in both species of Cys G11 α , Cys G14 β and Tyr H8 β (ref. 20). Of the thirty odd primates studied²¹, complete sequences are available for four and a partial one for another^{22–26}. The haemoglobins of the rhesus, capuchin and spider monkeys (all adult) contain little or no alkali-resistant haemoglobin and Cys G11 α , Cys G14 β and Tyr H8 β are all present. Oxyhaemoglobin of the brown lemur is denatured at pH 12.7 and 20° C at a rate similar to that of human foetal oxyhaemoglobin²¹; like the latter, it contains Cys G11 α but not Cys G14 β or Tyr H8 β (ref. 22). Oxyhaemoglobin of the slow loris is denatured at a rate intermediate between that of

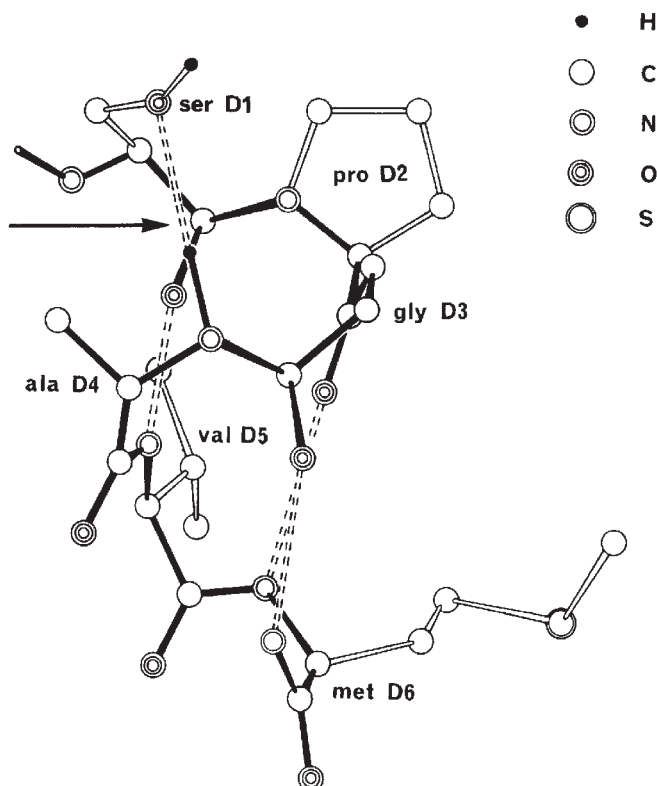


FIG. 2 Helix D with serine and proline at positions 1 and 2. The serine hydroxyl acts as a hydrogen acceptor to the main chain imino group of alanine D4. The hydrogen bond is marked by an arrow. Ionisation of the hydroxyl group would strengthen this bond.

human adult and foetal haemoglobin. Its sequence differs from that of the human adult form in only nine positions in the α and eleven in the β chain²⁵. All but four of the twenty replacements are external; one lies in a surface crevice and involves the replacement of Ala G18 α by Cys, but the side chain is so positioned that the S⁻ ion would be accessible to solvent and should not affect the rate of alkali denaturation. Another lies in a surface crevice at GH1 and involves the replacement of Leu by His which would both be uncharged at alkaline pH. Of the two internal replacements, one is Ser for Val at H6 β which would both be uncharged below pH 13 and the other is Val for Cys at G14 β . Cys G11 α and Tyr H8 β are both present. The same applies to rabbit haemoglobin^{22,27}, which also has a rate of denaturation intermediate between that of human and bovine.²⁸ Table 1 summarises these findings and shows them to be consistent with my interpretation.

Among other species sequenced so far Cys G11 α is absent only in bovine, sheep and goat haemoglobin, where it is replaced by serine. Cys G14 β is present only in primate and dog haemoglobin; the kangaroo has a cysteine buried in position G15 β , but this lies away from the $\alpha_1\beta_1$ contact²⁹. The sequences indicate that the high susceptibilities to alkali denaturation found in many adult primate haemoglobins and in the dog are exceptional among mammalian species.

Probable mechanism

In normal, homogeneous haemoglobins, the denaturation reaction follows first order kinetics^{9,28}. For several reasons, dissociation of the $\alpha\beta$ dimer into monomers is likely to be the rate-limiting step, followed probably by uncoiling of the chains from the carboxyl end.

The linkage between dissociation and denaturation in human adult haemoglobin has already been noted, but does not tell us which comes first. Haemoglobin Rainier (Tyr HC2(145) β $\rightarrow$ Cys) is a variant in which helices H and F are joined by a disulphide bridge^{30,31}. At pH 12.0 this haemoglobin dissociates into monomers and the normal α chains are denatured, while the abnormal β chains remain native³². At the same pH human foetal haemoglobin resists denaturation, even though its α chains are the same as those of Rainier. Apparently the presence of two ionisable sulphhydryl groups in the $\alpha_1\beta_1$ contact of haemoglobin Rainier and of only one in foetal haemoglobin causes dissociation of the dimer in the former but not the latter. The $\alpha_1\beta_1$ contact links mainly the G and H helices and the GH corners of the two subunits and, as long as it remains intact, it would oppose uncoiling of the chains from the C terminus but not from the N terminus. The same is true of the disulphide bridge which links residues 93 and 145 in haemoglobin Rainier. Uncoiling from the C terminus may be driven in part by ionisation of the penultimate tyrosines HC2. On the other hand, there are certain serines and threonines whose ionisation would actually stabilise the N termini of several helices at alkaline pH. These are external and occur in positions 1 or 2 of α helices; their γ hydroxyls are so arranged that they can act as hydrogen acceptors from the main chain imino groups four residues forward along the helix^{3,32} (Fig. 2). They include Ser A1, E1, F2 and Thr H1 β and Thr A1, Ser or Thr D1 and Thr H1 β or γ . Because the lone pair electrons of these hydroxyls act as hydrogen acceptors, their pKs should be less than the 13.60 measured in N-acetylserine amide. The ionisation of these hydroxyls above pH 12.0, say, would strengthen their hydrogen bonds with the imino groups and oppose uncoiling of the helices from the amino ends.

Figure 3 summarises the equilibrium mechanism, showing how dissociation of the $\alpha\beta$ dimer allows the sulphhydryl groups buried at the interface to become ionised and hy-

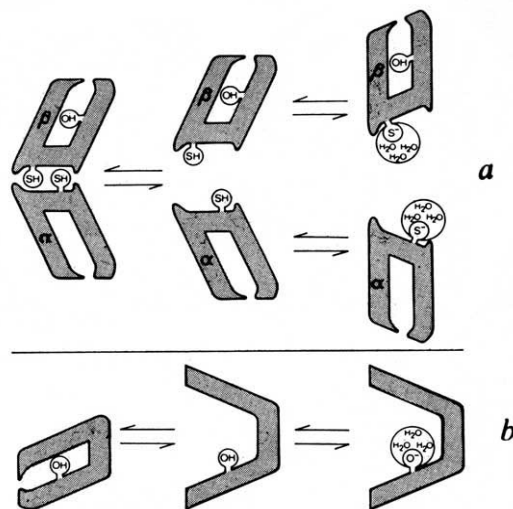


Fig. 3 Hydration effect of buried ionisable groups on the equilibrium between α , dimer and monomers; b, the native and denatured monomer.

drated; the bulk of the hydration shell opposes reassociation so that the equilibrium is shifted towards the monomeric form. Similarly opening of the tertiary structure of the monomer allows the buried tyrosine to become ionised and hydrated, and access of water into the nonpolar interior displaces the equilibrium towards the unfolded form. Alkali denaturation of human oxyhaemoglobin has an entropy of activation of 39 e.u., and bovine one of only 6 e.u. ref. 28, consistent with activation immobilising more water molecules in the human than in the bovine form. In the denaturation of monomers the electrostatic factor mentioned in the introduction may also play an important part, but buried charges speed the rate as the following example shows.

The myoglobins of chimpanzee and horse differ in eighteen positions; one of them is G11, buried between helices G and B, which is occupied by cysteine in chimpanzee³⁴ and by alanine in horse.³⁵ On reading a draft of this paper, Professor H. Lehmann wondered if this replacement would affect the susceptibility to alkali denaturation. He found that after 20 s at pH 12.7 and 20° C half of the cyanomet-myoglobin of the chimpanzee was denatured, but only 10% of that of the horse.

Denaturation of haemoglobin by acid is probably activated by ionisation of histidine G10 α which is buried in the $\alpha_1\beta_1$ contact and is invariant in mammals.

I thank Professor N. A. Barnicot, Professor E. R. Huehns, Dr A. R. Fersht and Mr P. A. Lorkin for drawing my attention to published data, and Dr B. S. Hartley for discussion on mechanisms.

Note added in proof. Since this article was submitted, Smith *et al.* have published an observation which bears on the problem discussed here³⁸. A human variant haemoglobin has a fused $\gamma\beta$ chain in which the N-terminal half is γ and the C-terminal half is β (ref. 39). It has the same high susceptibility to alkali denaturation as adult haemoglobin A, which proves that this property is determined by residues in the C-terminal half of the β chain.

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Paleomagnetic stratigraphy and chronology of hominid-bearing sediments east of Lake Rudolf, Kenya

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Palaeomagnetic measurements of the polarity of sediments from the Koobi Fora Formation east of Lake Rudolf provide a valuable check on other dating methods and allow a polarity stratigraphy to be suggested for the Formation.

THE Plio-Pleistocene sediments of the Koobi Fora Formation east of Lake Rudolf have yielded a rich assemblage of fauna, hominid fossils and stone artefacts. Here we report a study of the palaeomagnetism of the area, based on over two hundred samples collected under the supervision of one of us (G. Ll.I.) and measured in the Nairobi laboratory of the other (A.B.).

Stratigraphy and lithology

Figure 1 is a sketch of the area showing that the accessible outcrops occur along a strip of terrain between an uplifted lava plateau and the modern Lake Rudolf¹. The sediments rest on lavas, and a block of the underlying volcanics has been uplifted to form the Kokoi structure between Koobi Fora and Ileret. The outcrops of the Koobi Fora Formation with which this report is concerned, have a U-shaped configuration around the Kokoi horst. Palaeomagnetic samples

were taken from four stratigraphic columns at points around the U (Ileret areas 6 & 8, the Karari escarpment areas 130 and 131, the KBS Ridge—area 105, Koobi Fora area 103).

Figure 2 shows the columns which have been studied and also a series of tuff layers which are used as marker beds because they were apparently deposited rapidly over wide areas. Potassium-argon age measurements on several of the tuffs suggest that the most reliable determination is for the KBS tuff which yields an age of 2.61 ± 0.26 Myr (ref. 2). The KBS tuff can be recognised in all the main sections including those at Ileret, and it has been used by Bowen and Vondra as a marker for the boundary between the Lower and the Upper Member of the Koobi Fora Formation.

The sediments of the Lower Member are predominantly of lacustrine or lake-margin facies because at the time of deposition proto-lake Rudolf extended much further to the east. They consist of thin bedded clayey siltstones, and silty claystones, with interbedded sheets and lenses of moderately well sorted prodeltaic and littoral sands. The clayey siltstones are reasonably firm and comprise good material for palaeomagnetic sampling.

In area 105 a mappable disconformity has been recognised at a stratigraphic level just above the KBS Tuff³, and an analogous sharp lithological change with channelling also

occurs in area 130. In area 103, in a zone close above the KBS Tuff, there is an episode of tectonic disturbance followed by erosion and the influx of coarser sediments (unpublished work by G. D. Johnson). These disconformities are important for the palaeomagnetic interpretation which will suggest that they represent a time gap as large as 0.7 Myr.

In area 130, the Upper Member consists of comparatively high energy fluvial deposits, with cut and fill bedding in which coarse channel conglomerates and sands pass laterally into flood-plain silts. Only the latter could be used for palaeomagnetic sampling. The Upper Member in area 105 is similar although finer grained. In area 103 the Upper Member is

The initial directions were classified as normal, reversed, or intermediate (N, R, I). Intermediate directions were defined arbitrarily as those which departed from the geocentric axial dipole field axis for Koobi Fora ($D = 0^\circ, 180^\circ$, $I = \pm 8^\circ$) by more than 50° . The same classification was used for cleaned directions except that in a few cases the cleaning procedure revealed erratic directional changes due to instability. Such samples were described as showing no clear result (X). Table 1 shows the classification of samples before and after demagnetisation. Of the 247 samples, 75 changed their classification after demagnetisation.

The cleaned polarity results are shown on the sampling

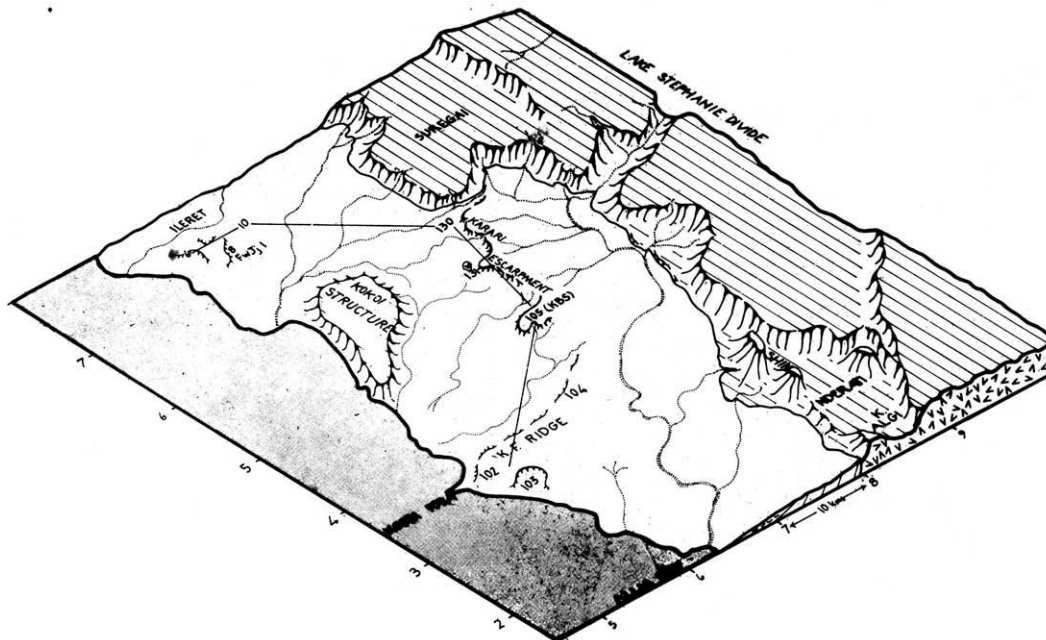


FIG. 1 An isometric view showing the relationship of the studied areas to the modern topography.

composed of siltstones, mudstones, sand and algal limestones. These represent deposition in littoral and lake margin environments.

At Ileret the sediments above the KBS Tuff are designated as a separate stratigraphic unit called the Ileret Member¹. The Upper portion of this, which was sampled palaeomagnetically, consists of very low energy fluvial silts and sands with at least one lacustrine incursion towards the top of the section.

Palaeomagnetism

Slumped and weathered material was removed, and non-magnetic tools were used to carve the sediment into small rectangular pillars to fit an open plastic cube of side 2.4 cm. The cube was placed over the pillar and oriented with a compass and inclinometer. The sediment filled cube was then detached and the open side closed with a plastic lid. Wherever possible three or four samples were taken at each horizon, with a spacing of a few metres. Siltstones with varying admixtures of clay or sand were found to be suitable for collection in this way; the coarser grained sediments proved difficult to sample and were avoided. Fortunately, suitable material was abundant in all sections and a total of 247 samples was collected.

The oriented cubes were measured using a 5 Hz spinner magnetometer of the "Foster" type⁴, and subjected to the usual a.c. demagnetisation procedures. In most cases secondary components were removed by partial demagnetisation in peak a.c. fields of 100 oersted. Typical demagnetisation curves and directional changes are shown in Fig. 3. An analysis of the directions for information about secular variation, and a study of the magnetic properties of the samples are in progress: here we report solely the polarity results.

columns in Fig. 2. The polarities at each sampling horizon are generally consistent. The occasional discrepancies could be due to orientation errors, hard secondary components not removed by cleaning, or discordant ages of magnetisation. Nevertheless the polarity sequence within the columns is generally clear, and the results in Fig. 2 provide the raw

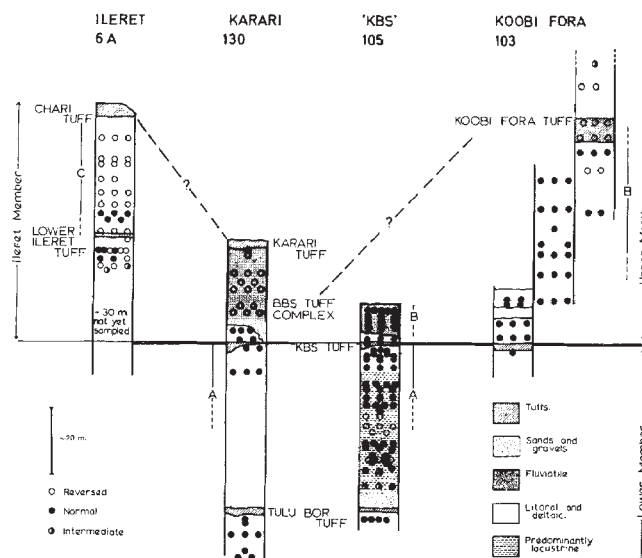


FIG. 2 The sampled columns and the cleaned palaeomagnetic results. Maglio's faunal zones⁷ are as follows: A, *Mesochoeus limnetes*; B, *Metridiochoerus andrewsi*; C, *Loxodonta africana*. ○ Reversed polarity; ●, normal polarity.

TABLE 1 Classification of samples before and after cleaning

		After cleaning			
		N	R	I	X
Before cleaning	N	129	16	8	17
	R	0	40	1	0
	I	10	20	3	3
		139	76	12	20
					247

material for making correlations with the geomagnetic polarity time scale. Figure 4 shows the correlation favoured by us. The time scale is based upon that of Cox⁵ but the polarity intervals are named according to the scheme of Grommé and Hay⁶. The correlations shown in Fig. 4 are not fully independent, and rely partly upon K-Ar and faunal evidence as well as upon the basic polarity data.

Polarity stratigraphy and the K-Ar and faunal evidence

The starting point for the correlation is the age of 2.61 ± 0.26 Myr obtained by Fitch and Miller² from selected sanidine crystals from pumice specimens from the KBS Tuff. The polarity of samples taken from, in, or near the KBS Tuff is everywhere N which is consistent with the quoted age, and in the correlation diagrams the KBS Tuff is placed at 2.61 Myr in the Gauss normal epoch.

The polarity of the Lower Member from the KBS Tuff downwards is predominantly normal, but in area 105 two short reversed intervals occur in a stratigraphic position which suggests that they represent the Kaena and Mammoth events. The sampled part of the Lower Member thus appears to have been deposited entirely during the Gauss normal epoch. In area 105 the spacing of events in relation to each other and to the KBS Tuff implies a net accumulation rate of about 10 cm in 10^3 yr. A similar rate can be anticipated in area 130 and in area 131 (not sampled) which are of similar facies and which show a similar thickness between the KBS Tuff and the Tulu Bor Tuff (50 m).

An independent chronology for the Koobi Fora Formation has been presented by Maglio⁷ on the basis of mammal fossil zones, and it is interesting to compare these with the palaeomagnetic results (Fig. 2). Maglio's *Mesochoreus limnetes*

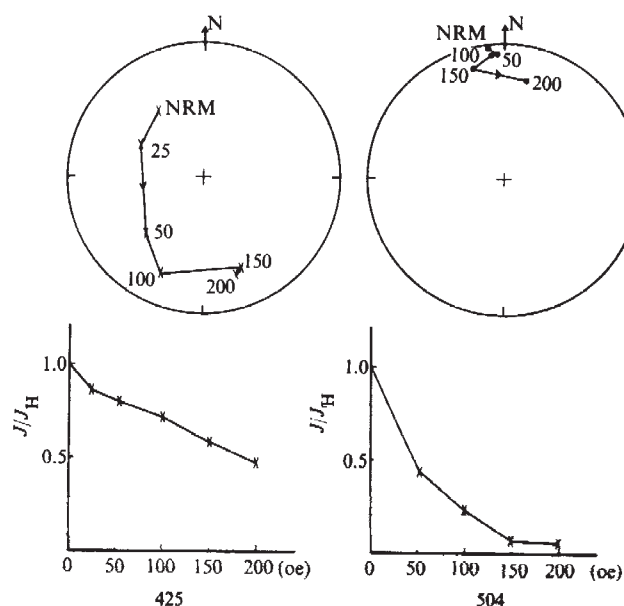


FIG. 3 Typical demagnetisation curves and directional changes for samples 425 (N → R) and 504 (N → N). Secondary components are largely removed by 50 oersteds.

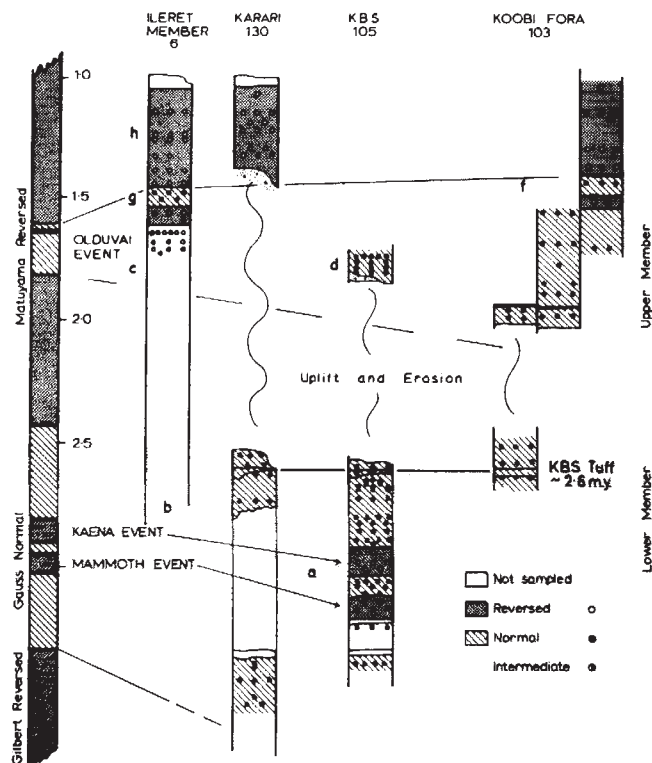


FIG. 4 Tentative correlation of the polarity results with the geomagnetic polarity time scale. The letters a-h refer to the groups of hominid fossils in Table 2.

zone (A) is defined by an assemblage of fossils from the upper part of the Lower Member which has normal polarity. A 'best fit' age of 2.3 Myr with a range of uncertainty from 3.0 to 2.0 Myr is suggested for this zone on faunal grounds. The palaeomagnetic evidence requires an age greater than 2.43 Myr and in conjunction with the K-Ar determination for KBS Tuff it suggests that the age for the center of the *Mesochoreus limnetes* zone be revised upwards to about 2.7 Myr.

The fossils used to define the second, or *Metridiochoerus andrewsi*, zone (B), come from outcrops of the Upper Member in which the polarity is predominantly normal. Maglio's estimate for the age of this zone is 1.7 Myr with a range from 1.9 to 1.5 Myr which would imply that the normal polarity belongs to the Olduvai Event. The recognition of a disconformity just above the KBS Tuff in both areas 103 and 105 fits this interpretation and implies a span of time unrepresented by sediments, of the order of 0.7 Myr. The ragged upwards transition from normal to reversed in area 103 would then mark the end of the Olduvai event.

Maglio's third faunal zone, *Loxodonta africana* (C) is represented only in the Ileret Member above the lower tuff, and has an estimated age of 1.3 ± 0.3 Myr. The palaeomagnetic column starts just below the Lower Tuff with a rather mixed polarity pattern, changing upwards into a consistently reversed section to which the main fossil assemblage belongs. Until this column is extended further downwards its placement in the reversal chronology must remain conjectural, but it seems likely that the magnetically complex zone represents the end of the Olduvai event, with the reversed beds above being in the post-Olduvai part of the Matuyama. This would be in accord with the faunal age.

The Upper Member in area 130 gave consistently reversed polarities from the local disconformity up to the Karari Tuff. Few mammal fossils have been recovered from these beds, although they do contain many archeological sites. Since there are grounds for correlating the Karari Tuff in area 130

ology.

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Magmatism, orogenic timing, and orogenic diachronism in the Cordillera from Mexico to Canada

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The distinction between the largely intra-Cretaceous Sevier orogeny and largely post-Cretaceous Laramide orogeny in the Cordillera between Montana and New Mexico does not exist in the northern Rocky Mountains of Montana and Canada and in the Cordillera of Mexico. An explanation of this in terms of the tectonics of the region is proposed.

THE region of high heat flow, and consequently thin lithosphere, behind active volcanic arcs is of profound importance in the tectonic evolution of continental margin orogenic belts. Armstrong and Dick¹ discuss how overthrust sheets of crystalline rocks are dependent for their genesis on such regions of anomalous heat flow, and it is possible to adopt the even more generalised hypothesis that crustal weakening in zones of anomalous heat flow is a necessary precondition for any sort of tectonic deformation resulting in a large-scale crustal-shortening strain within lithosphere plates. Normal lithosphere (whether continental or oceanic and far from rises or hot spots) with a near-surface thermal gradient of less than 30° C is presumably exceedingly difficult to deform by compression. Stresses within the lithosphere will find their release along plate margins and in weaker zones whenever possible, and any region of high heat flow is obviously vulnerable.

In the Cordillera of western North America, nature has performed a grand, controlled experiment to test the hypothesis that behind-the-arc heat flow controls orogenic deformation. A fortunate by-product of this viewpoint and discussion is an explanation of tectonic diachronism along the Cordilleran fold and thrust belt that separates the flat-lying strata of the craton from the more complex structural belts that lie further west.

The problem of diachronism and a possible explanation have come from two seemingly divergent directions of research—radiometric dating of Mesozoic and earliest Tertiary

igneous rocks related to volcanic arc activity, and stratigraphic bracketing of deformation in the fold and thrust belt, which for most of its length lies east of and is geographically distinct from the shifting belt of igneous activity. Here I am not concerned with the complex and incompletely deciphered events of more recent date than early Eocene, the time when a major change in Cordilleran tectonic patterns occurred².

King³ recognised the significant changes along regional strike in the Cordillera and proposed a subdivision into segments. In the area discussed in this paper he recognised four such segments: Mexico, California-Colorado, Oregon-Montana, and Alaska-Canada. The boundaries of the California-Colorado (C-C) segment are shown in Figure 1. For my purposes it is only necessary to distinguish that segment from areas to the north and south.

The obvious distinction of the C-C segment is the eastern Rocky Mountains—uplifts of Precambrian basement bounded by steep, reverse, and thrust faults. Thin sediment cover here was deformed in passive response to movements of the crystalline basement or became detached in gravity-driven slide masses. These Laramide structures, including the Laramie Range itself, can be precisely dated because intervening basins preserve a complete record of their evolution; from first appearance very late in Cretaceous (late Campanian, ~ 72 m.y. ago) time to end of active rise in early Eocene time (~ 52 m.y. ago)⁴. Contrasting in time of deformation and tectonic style is the Sevier orogenic belt⁵⁻⁷ of this same sector where miogeosynclinal sediments were deformed by low angle thrust faults and folds of décollement style. Preservation of orogenic elastic rocks in Utah and Wyoming makes possible the dating of most of the fold and thrust structures as Cretaceous. Movement began possibly as early as latest Jurassic time (145 to 150 m.y. ago) and had largely ceased in central Utah by late Cretaceous (late Price River—late Campanian, ~ 75 m.y. ago) time, although some deformation persisted into the Palaeocene, resulting in folding of conglomerates of the Flagstaff Formation. Likewise, most of the deformation in Wyoming preceded deposition of the Evanston Formation (Maestrichtian, ~ 65 m.y. ago) although some modest displacement on thrusts did continue

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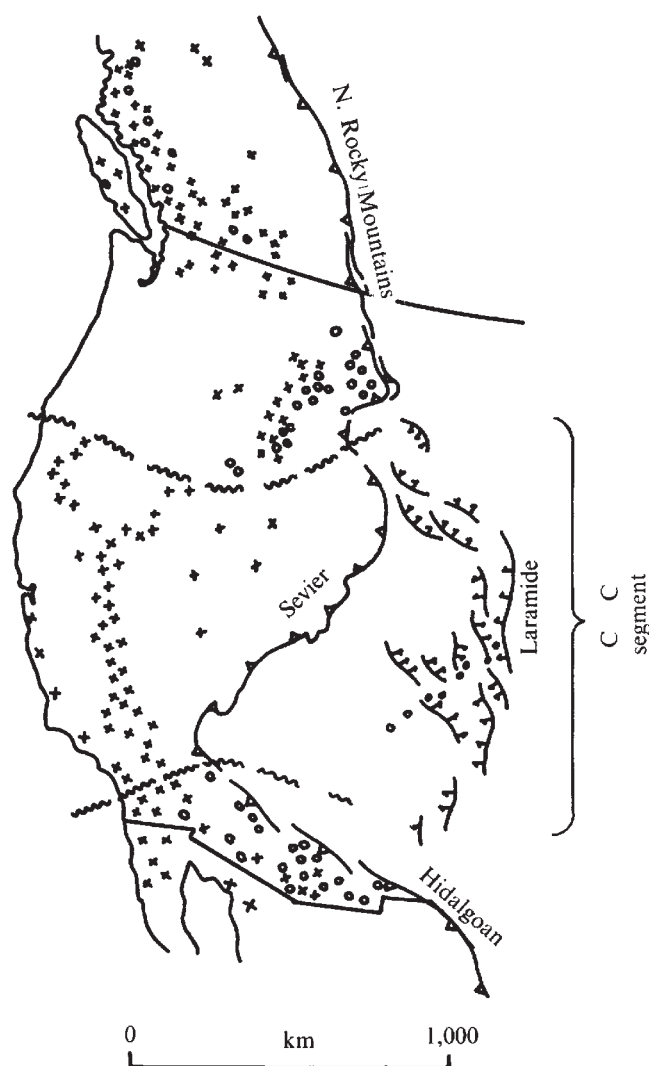


FIG. 1 Index map of central western North America showing the extent of the California-Colorado segment (C-C) and structural belts discussed in the text. X, Generalised distribution of Mesozoic plutons older than 80 m.y.; O, plutons younger than 80 m.y. but older than 55 m.y. Plutonic rocks are shown as they are the prime source of information on the distribution of Mesozoic igneous activity. A one-to-one correspondence between pluton emplacement and volcanism appears to have been true³¹ but the preservation of the volcanic record is poor due to erosion. Segment boundary shown as wavy line.

during Palaeocene and up to middle early Eocene time (~ 53 m.y. ago)⁸.

This contrast of Sevier and Laramide orogenies does not exist outside of the C-C sector. It has been traditional to apply the term Laramide to the period of deformation of the northern Rocky Mountains in Montana and Canada. This is obviously at variance with the usage of the term in the C-C sector. In the northern Rocky Mountains belt of folds and thrust faults, precise determination of the age of individual structures has been a more frustrating exercise than in Utah-Wyoming. A popular idea was that most of the deformation was Eocene, and this conclusion was emphatically stated in the literature up to the mid-1960s⁹⁻¹⁴. The available evidence in southern Canada was that strata up to Palaeocene in age were deformed in the Foothills belt; deformation of the Fernie basin in the Front Ranges had to postdate lower Cretaceous time and at least 50 km of displacement is younger than Belly River (lower Campanian ~ 78 m.y. ago) time^{15,16}.

A marked break separates Palaeocene clastics from upper

Eocene to Oligocene post-orogenic deposits, and this was considered by many to be the mark of an orogenic climax.

Acutely aware of the contrast in types of evidence used for dating orogeny in the Rocky Mountains of Canada and the Sevier orogenic belt and the conflicting conclusions reached in the two areas, Price and Mountjoy¹⁷ adopted the position that the Rocky Mountains in Canada were tectonically active from Upper Jurassic to Eocene time. The Jurassic to Palaeocene clastic wedges were interpreted to be a direct record of tectonic activity as the mountains grew and the deformation front moved eastwards—the deformation of Palaeocene strata being the last act rather than the result of a brief catastrophic orogeny in Eocene time. This view put interpretations of tectonic chronology in different segments of the Cordillera more in accord and solved inconsistencies in the Canadian picture such as evidence of lower Cretaceous structures in the western Main Ranges¹⁷.

Evidence of orogenic timing in Montana, obtained during the past decade, is in accord with Price and Mountjoy's view¹⁷. Most of the deformation of Palaeozoic and Mesozoic strata, including local thick accumulations of Upper Cretaceous volcanic rocks, had taken place before the emplacement of the Boulder and Phillipsburg batholiths (73 to 79 m.y. ago, $\sim$ Campanian)¹⁸⁻²⁰. A little further east the deformation occurred even later—after Adel volcanism (Maestrichtian, ~ 65 m.y. ago)^{21,22}, and after emplacement of late Cretaceous and sills in the Sun River area²³; movement occurred along the Eldorado thrust after Palaeocene deposition, but prior to intrusion of a 58.3 m.y. sill²⁴. In south-westernmost Montana the Upper Cretaceous to Palaeocene Beaverhead Formation was deposited in an area where Laramide style basement uplifts and Sevier style thrusts were concurrently supplying orogenic clastics²⁵⁻²⁶. Most of the deformation of the Beaverhead itself was during the Palaeocene. In the Yellowstone Park region of Idaho, Montana, and Wyoming rocks as old as upper Lower Eocene (~ 50 m.y. old, perhaps as old as 53 m.y.), the Challis and Absaroka volcanics, are clearly post-orogenic (refs 27 and 28 and R.L.A., unpublished).

From this brief review it should be evident that fold and thrust belt deformation is diachronous along as well as across regional strike. Although the Sevier and Canadian Rocky Mountain deformations have beginnings that are obscurely dated but seem similar and although the derived clastic wedges show astounding parallelism of development, their terminal phases do not match, regardless of how the data are manipulated or stretched. Deformation involving tens of kilometers of shortening occurred in Montana and Canada, while the movements in Utah and Wyoming were dying out. The orogenies cannot be equated or used as synonyms. If anything is true, Canadian Rocky Mountain orogeny equals the Sevier plus Laramide orogenies in time. A significant shift in locus of crustal strain that took place in the C-C sector about 75 m.y. ago did not occur in the sector to the north.

The record of orogeny in the Mexico segment, likewise, does not match that in the C-C sector. The main deformation may even be slightly different in age from orogeny in Canada but the available dating information is not accurate enough to prove that point. Deformation in western Mexico is known to have occurred during much of Mesozoic time, but an exact chronology is not known. A late Cretaceous to Palaeocene clastic-filled foredeep, which is analogous to the foredeep in the C-C and Canadian segments, covered much of eastern Mexico. The orogenic structures supplying the clastics have not yet been identified and named. These clastic deposits were folded in the Hidalgoan orogeny^{29,30}, which is considered to be early Eocene in age (roughly equated with Laramide). Post-orogenic deposition began in mid-Eocene time.

The Hidalgoan orogenic style is that of a fold and thrust belt. The effects of this orogeny are widespread, all of eastern and southern Mexico being involved. Here as in Canada, Sevier-style orogeny continued through Laramide time. The

abrupt eastward shift of the zone of deformation occurred about 60 m.y. ago, apparently not synchronous with major shifts in tectonic pattern in the more northern segments.

At least in the C-C sector (where the necessary documentation is most complete) it is possible to argue for an action-response relationship between magmatic activity in the volcanic-plutonic arc along the continental edge and deformation of the miogeosyncline. Armstrong and Suppe³¹ reviewed the magmatic history of the western United States south of 42° N. Acceleration, if not a new beginning, of igneous activity occurred in Upper Triassic time and activity persisted through the remainder of the Mesozoic, reaching a leisurely culmination in the late Jurassic-earliest Cretaceous and a more pronounced culmination in Middle Cretaceous time.

Except for the linear chain of Laramide volcanic centres that diagonally crossed the Colorado Rocky Mountains, magmatic activity in the C-C segment terminated abruptly about 80 m.y. ago.

Soon after each upsurge in magmatic activity in the Andean-type volcanic arc that stretched for thousands of kilometres parallel to the continental margin, deformation advanced eastwards, engulfing ever more of the previously stable miogeosyncline and platform. The upper Triassic beginning of magmatism was followed by orogenic deformation in western Nevada-southern California dated as pre-Upper Jurassic in the Inyo Mountains^{32,33}, as pre-Lower Jurassic in the Clark Mountains³⁴ and as pre-Upper Jurassic in northern Nevada³⁵. The Upper Jurassic and mid-Cretaceous maxima of igneous activity correspond to the beginning and most intense phases of the Sevier orogeny, respectively, and were synchronous as well with blueschist metamorphism and subduction in coastal California³⁶. The abrupt cutoff of magmatism about 80 m.y. ago in the Sierra region was soon followed by the shift from Sevier to Laramide styles of deformation. Rather than coincidence, this is thought to be a cause-effect relationship. Presumably, after igneous activity of the arc had ceased, the anomalously high heat flow that existed behind the arc died out. One could even imagine that the cutoff effect would migrate away from the volcanic front as the response of deeper zones would be more sluggish than shallower ones. Without the high heat flow, the lithosphere behind the arc would thicken, become more resistant to deformation, and orogenic deformation would cease. This would explain the end of the Sevier orogeny, but what about the Laramide and continuation of Sevier-style deformation in areas north and south of the C-C segment?

This is nature's controlled experiment. Both north and south of the C-C sector igneous activity persisted until 10 to 20 m.y. later, the 'Laramide' activity of Arizona-New Mexico and Mexico continuing on into the earliest Tertiary (until ~ 55 m.y. ago) and activity in Idaho-Montana and Canada dying out about the end of the Cretaceous (~ 65 m.y. ago). In both areas where magmatic activity continued unabated, so did Sevier-type orogenic deformation. Only in the C-C sector with its early magmatic cutoff did the change from Sevier to Laramide styles occur.

Exactly what caused the Laramide deformation is still not completely explained, but the discussion and model experiments of Sales³⁷ make it clear what happened in Colorado and adjacent states was compression, buckling, and shear that deformed the whole crust. The deformation style expresses much less ductility of the lithosphere than is evident in the hinterland of Sevier-style belts. The magnitude of crustal shortening actually evident in the Laramide structures is vastly less than that which seems to have occurred synchronously in the Cordilleran segments that lay to the north and south.

The Laramide structures represent the reluctant and only partial response to stress imposed on the C-C segment by continuing Sevier-style deformation in the Canadian-Montana

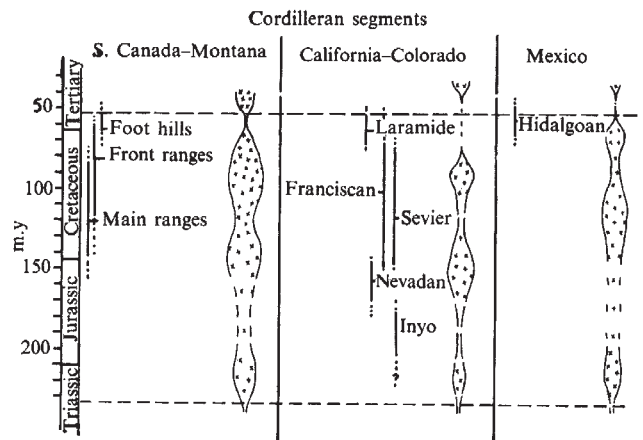


FIG. 2 Summary of chronological information as discussed and cited in the text; duration of orogenic deformation shown by dark lines, arc magmatism of varying intensity suggested by areas with cross pattern. Time scale from Armstrong and Suppe³¹.

and Mexico segments. Premature cutoff of arc activity in the C-C segment (an observation for which a precise plate tectonic explanation is not yet available) made it no longer possible for Sevier-style crustal shortening to occur. Compressive stress continued however, and found its release in the Laramide style of orogenic strain. By late Lower Eocene time, ~ 53 m.y. ago, a shift to an altogether different tectonic pattern had taken place in the C-C-Montana-Canada segment; the change in the Mexico segment came soon after that.

The change from subduction to a different tectonic style in coastal California occurred in early Tertiary time about the end of the Laramide orogeny, but there is some disagreement among geologists working there as to the exact dating of this event: post-Maestrichtian, pre-mid-Eocene³⁸; pre-Oligocene³⁹; post-Palaeocene and early Eocene⁴⁰; post-Maestrichtian, pre-late Palaeocene-early Eocene⁴¹. A portion of the uncertainty may derive from diachronism along strike just as observed in the fold and thrust belt. Subduction that occurred after Santonian time was evidently insufficient to maintain volcanic arc igneous activity. Whether these late stages of movement were intermittent or merely slow and leisurely is yet unknown. What is significant is that compressional tectonics ceased on both sides of the Cordilleran orogen approximately simultaneously.

To summarise briefly (Fig. 2), we have described how the C-C sector of the Cordillera is unique in having overlapping yet distinct Sevier and Laramide orogenies on its eastern side and likewise unique in an early termination of Mesozoic volcanic-plutonic arc magmatic activity in a zone parallel and closer to the west coast. This suggests a cause and effect relationship between an active volcanic arc of Andean type and the orogenic zone far inland from the arc. This is presumably because high heat flow behind the arc weakens the lithosphere so that it may be compressed or stretched apart⁴², depending on plate motions dictated by processes acting on a global scale⁴³. The anomalous heat flow is not itself a cause of deformation, just a control on where and when it will occur.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Is Cyg X-1 a neutron star?

We suggest that the source of X-ray emission Cygnus X-1 may be a neutron star in orbit about an early-type relatively unevolved star, both of which are in orbit about the massive 09.71ab (ref 13) supergiant star DH226868. From an analysis of HD226868, Bolton¹ derives the following parameters for the system: $M_1 \sim 20M_\odot$, $M_2 \sim 11M_\odot$, $A \sim 3 \times 10^{12}$ cm, $i \sim 30^\circ$, $P \approx 5.6$ d, $L \sim 10^{39}$ erg s⁻¹ and $T \sim 30,000$ K, where M_1 and M_2 are the masses of the primary and secondary components respectively, A is the separation of the centres of the components, i is the inclination, P the orbital period, L the luminosity and T the effective temperature. It has been suggested by numerous authors (for example refs 1-3) that the source of the X radiation is the unseen secondary, which, since it is massive and since it must be compact to produce X rays, is likely to be a black hole.

The alternative hypothesis that Cyg X-1 = HD226868 is a triple system is worth examination. Indeed Batten⁵ has noted that about a third of observed binary systems are in fact triple. Specifically we consider the parameters mentioned above and suggest that the secondary consists of two stars—a $1M_\odot$ neutron star (the source of the X rays) and a $10M_\odot$ B1V star with radius $R \sim 3 \times 10^{11}$ cm, luminosity $L \sim 3 \times 10^{37}$ erg s⁻¹ and temperature $T \sim 25,000$ K (ref. 6) (see Fig. 1). Assuming, for simplicity, that the orbits are coplanar, limits can be placed on the size

of the neutron star's orbit around the B1V star. Because of the absence of regular X-ray occultations we must demand $9 \text{ h} \lesssim P_N \lesssim 3.3$ days, where P_N is the orbital period of the neutron star. For stability of the system, however, we need the ratio of the radii of the orbits to be more than ~ 3.5 (ref. 7) which implies $P_N \lesssim 1.4$ d.

The optical light curve of this triple system is comprised of two main components. First, there is an underlying ellipsoidal variable contribution, with period $P = 5.6$ d and amplitude of about 0.05 mag in the B band, caused by the distortion and rotation of HD226868 (refs 8 and 9). Second, there is a contribution with periods P and P_N due to the reflection effect (X-ray heating) on both stars. The strength of this modulation depends on the sizes of the orbits and on the total intensity of the X-ray source, but can be comparable to the ellipsoidal effect. Such possibilities can qualitatively explain the anomalous light curve behaviour found by Walker⁴ and Martynov¹⁰ especially with regard to the occasional 'filling in' of the secondary minimum.

We now discuss further predictions of the model. Due to the triple nature of the system we expect the radial velocity curve of HD226868, with period P and amplitude ~ 70 km s⁻¹, to contain a superimposed variation with period P_N and amplitude $\ll 1$ km s⁻¹. The present data are of insufficient accuracy to detect this.

Since the two non-degenerate stars are both early-type, they may both have strong enough stellar winds to power the X-ray source. If the supergiant provides most of the mass transferred, the intrinsic luminosity of the X-ray source would vary in a regular manner with period P_N , unless the time taken for accreted material to lose its angular momentum via the accretion disk is much larger than P_N . In addition, absorption by both stellar winds may

provide a quasi-regular modulation of the softer end of the X-ray spectrum¹¹ with predominant period P or P_N , de-

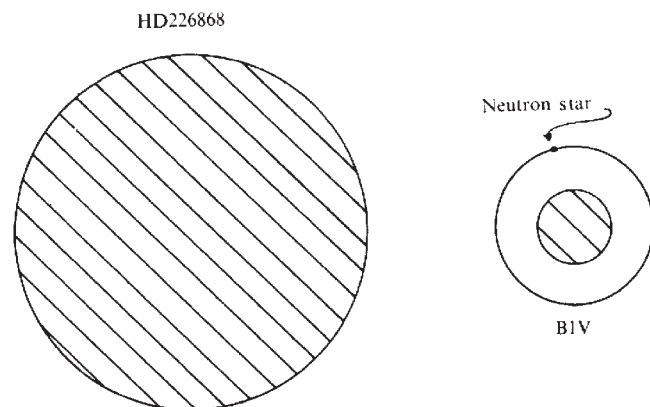


FIG. 1 The system Cygnus X-1 = HD226868 drawn to scale (except for the neutron star). Tidal effects have been ignored.

pending on the relative importance of the winds. The overall temporal behaviour will be complicated by irregularities in the winds and the presence of gas streams.

The X-ray flux from Cygnus X-1 is observed to vary on short (a few milliseconds) timescales¹², but with no regular period. Accretion by a neutron star need not give rise to any regular pulsation, for example if the star's magnetic field is weak ($\lesssim 10^9$ gauss) or if the rotation and magnetic axes are aligned³. We note that weak pulsation would be relatively difficult to detect due to the possible high orbital velocity of the neutron star ($\sim 500 \text{ km s}^{-1}$ if $P_N \sim 9 \text{ h}$). We emphasize too that the shortest timescales of variability to be expected from matter accreting onto a $10M_\odot$ black hole or a $1M_\odot$ neutron star are comparable.

We conclude that there is, as yet, no need to invoke the existence of black holes to explain the properties of X-ray sources.

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Note added in proof: Professor M. J. Rees has recently drawn our attention to a preprint by Professor J. N. Bahcall and colleagues at Princeton which discusses the possibility of Cyg X-1 being a triple body system and reaches similar conclusions to our own.

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Seismic Wave Scattering Near Caustics: Observations of PKKP Precursors

ANALYSIS of precursors to PKP^{1,2} has provided evidence for the existence of scattering structures in the deep mantle. It has been argued that the observability of the scattered waves is closely related to the presence of a caustic because of the focusing effect and because the scattered waves can enter the shadow zone at the concave side of the caustic, where they may manifest themselves as first arrivals. If that interpretation is correct, then in suitable circumstances the scattering phenomenon should also be identifiable near other caustics. The Earth's core forms a caustic not only for PKP phases, but also for SKP, PKKP, SKKP etc. and thus there is, in principle, the opportunity to test the interpretation. In practice there are a number of limitations: first, the latter phases themselves are one or more orders of magnitude smaller than PKP; second, at the time of arrival of these phases the seismic traces are, in general, still 'noisy'. This practically excludes identification from single traces. We have used data from the Norwegian Seismic Array (NORSAR) and here observations in the shadow of the PKKP caustic will be reported. The PKKP caustic agrees mostly with the one of PKP; both caustics extend throughout the whole mantle, in contrast to those of, for example, SKP and SKKP.

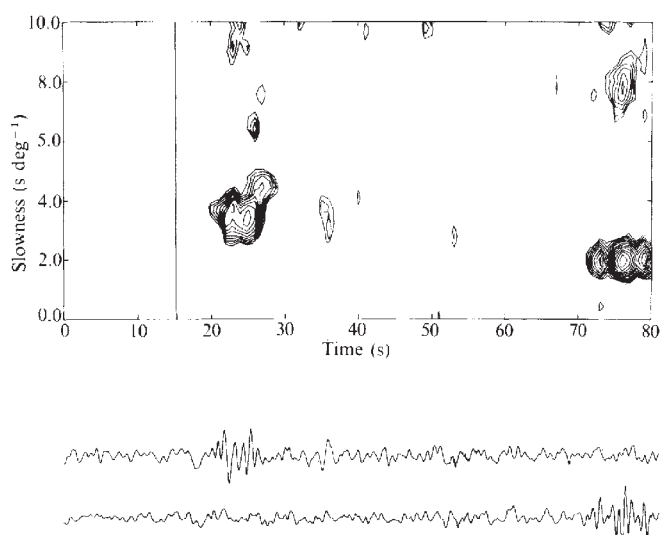


FIG. 1 A Vesogram processed to record PKKP phases from an event with origin time=October 27, 1971, 17 h 58 min 36.9 s, magnitude=6.0, depth=40 km. Epicentral distance from NORSAR=131.6°. The Vesogram start time is at 18 h 26 min 30 s. Power is contoured from 1 to 12 dB below peak power, which is at 77 s. The vertical line at 15 s represents minimum arrival time for PKKP scattering from this event. The traces below the Vesogram represent the NORSAR beams directed towards the PKKP peak (lower trace) and its peak precursor (upper trace).

In detection and identification problems like that it is often advantageous^{3,4} to form a display of signal energy versus $dT/d\Delta$ and travel time, called a Vesogram. A typical Vesogram is shown in Fig. 1. It is processed to pick out the PKKP phases from an event at an epicentral distance of 131.6°. The PKKP caustic at the Earth's surface is near 125° and a shadow region for PKKP outer core phases extends from 125° to larger distances, only PKKP which is reflected by and/or refracted through the inner core will be expected here. In the Vesogram it is easy to identify as the inner core phase the peaks in the end of the time window at about 2.0 s deg⁻¹, and it is clear then that there are one or

more precursors, starting about 50 s before PKKP. As indicated in Fig. 1, the minimum travel time for scattered waves is at about 15 s after Vespagram start time.

PKKP with its precursors from eight events, all in the Solomon Islands region, have been analysed this way. In the Vespa process the array is steered in a fixed azimuthal direction, which for PKKP is taken the azimuth away from the event (the 'back azimuth'). Since scattered waves may arrive from a variety of azimuths, the maxima in the Vespagrams have been relocated in frequency-wave number space, using the spectral analysis method from ref. 2. Several of the events were fairly complicated, which will cause large arbitrariness in interpreting later arriving precursors. Therefore, in Fig. 2 only results for travel time and direction of approach of the first arriving precursor of each event have been given. In Fig. 2a the travel time differences with PKKP have been used, they are plotted along with the PKKP branches from a model of Buchbinder⁵. With these results the following comments can now be made.

(1) It may be remarked that interpretations of PKP precursors in terms of layering in the outer core also predict PKKP precursors. But whereas these interpretations were made to contain the travel times of precursors to PKP, the corresponding PKKP precursors will be 5 to 10 s late with respect to the arrivals in Fig. 2a. Thus the 'layering hypothesis' leads here to a discrepancy not only in $dT/d\Delta$ (this would be below 3.3), but also in travel time.

(2) In our working model, $dT/d\Delta$ of PKKP at cusp B is about 4.18 s deg^{-1} . Pursuing now the scattering hypothesis,

phases with larger $dT/d\Delta$ are most likely to be scattered in the deep mantle at the source side of the PKKP path, and scattering characterised by smaller $dT/d\Delta$ should be generated at the receiver side (see ref. 2). With such a model, the travel times are reasonably consistent with the slowness vectors, with a discrepancy of several seconds in only two cases. With one exception, scattering within a few hundred km from the core-mantle boundary can explain the data. This is of course to be expected for first arrivals. Besides, it was argued in ref. 2 that deeper structures are more likely to be revealed, since the direction of scattered waves into the shadow will deviate increasingly from the primary wave direction, if the source of scattering is moved upwards from the core-mantle boundary. Here, this tendency is reinforced by the transmission of PKKP through the core. Although the details of the core-mantle boundary, and especially the density ratio, are still not well known, all plausible models give a high transmission only for PKKP with near grazing incidence from the mantle into the core, which is mainly due to the reflection at the core-mantle boundary within the core. This effect was also recognised in observations of phases which are multiply reflected within the core (PKKKP and so on)⁶. With core phases limited within a ray bundle near grazing incidence, the aforementioned direction deviations of scattered waves will become much more pronounced. Knowing also that the focusing of core phases in this ray bundle increases with depth, the result will be to restrict the source for PKKP precursors further to regions mainly near the core-mantle boundary.

(3) With the limited number of data and the known restrictions for PKKP scattering, inferences on the structure are bound to be very speculative. All we can say at this stage is, that the variations in $dT/d\Delta$ and the relatively small azimuth deviations of most precursors give little indication for lateral variation in the scattering structure. Lateral variations have been inferred from observations of PKP precursors² and, though not established, it might be possible that there is a correlation with structural variations suggested by characteristics of direct P waves⁷⁻⁹. But those variations are not necessarily manifest in the limited region, which could effectively be 'seen' with the given configurations of sources and receiver. We finally mention that the only precursor with a relatively large azimuth deviation in Fig. 2c was also the only one whose scattering source could not be near the core-mantle boundary.

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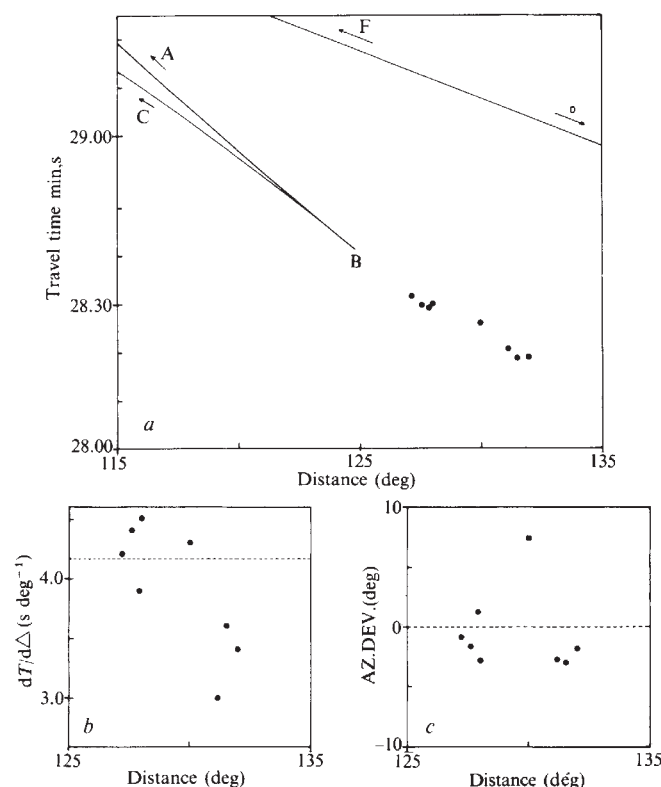


Fig. 2 Travel times and slowness vectors of first arriving PKKP precursors. The distances adjusted to surface focus. a, Observed travel time differences with PKKP referred to PKKP branches from a model of Buchbinder⁵; b, $dT/d\Delta$ (the line at 4.18 s deg^{-1} represents $dT/d\Delta$ at cusp B for the reference model); c, differences between the azimuth of approach and the back azimuth of the event. These differences are called azimuth deviations.

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Age of Fault Movements in Tanzanian Sector of East African Rift System

THE Neogene volcanic province of northern Tanzania is a southerly extension of the more extensive volcanic areas of Ethiopia and Kenya. It stands astride the Eastern Rift Valley and represents a complex interplay of volcanic activity and widespread Earth movements with associated faulting. A general picture is that an older series of basaltic-trachytic shield volcanoes, together with smaller nephelinitic centres, erupted and grew up on the floor of a major late-Tertiary fault-bounded depression; this earlier series includes the basaltic volcanoes of the Crater Highlands, Oldoinyo Sambu, Gelai and Kitumbeine, and the nephelinitic volcanoes Mosonik and Essimigor (Fig. 1). This volcanic area was later disrupted by a major episode of faulting that created the present-day Rift Valley depression. The main manifestation of this faulting is the major Rift Escarpment that runs from Lake Natron in the north to well south of Lake Manyara, this escarpment being the surface expression of the major Manyara-Natron Fault. The Tanzania sector of the Rift Valley is not a classic graben, as in Kenya to the north, and is bounded on its eastern side only by minor faults or down-warping. The major faulting was followed

by another phase of volcanic activity, the volcanoes being dominantly nephelinitic, with carbonatite present at some centres; the major volcanoes are Hanang, Ufiome, Meni, Burko, Kerimasi and Oldoinyo Lengai together with many smaller volcanoes¹. Previous geochronological studies on volcanic rocks from the Tanzania province have outlined the ages of various volcanic centres², and also of individual flows in connection with palaeomagnetic determinations³ or anthropological studies⁴. The work described here has been directed at dating the faulting episodes, particularly the main phase, with a view to setting a maximum age for the onset of the later nephelinite-carbonatite magmatism, and also for the comparison with dated volcanic areas in Kenya to the north^{5,6}. Such basic age information is also necessary for examination of the suggested temporal and spatial association of swelling, rifting and volcanicity (pluming?) with carbonatite magmatism⁷.

Geological observations suggest that the Manyara-Natron Fault results from two episodes of faulting acting along the same plane of weakness. In the Manyara-Engaruka area, James⁸ has recognised an early minor phase—now represented by a mature erosion level at the top of the escarpment—and a later major episode reflected by the steep, less mature lower part of the escarpment. Further north, on

Table 1 Analyses of Rocks Selected from Locations given in Fig. 2

	Sample No.	Locality	Map ref.	K (wt %)	$^{40}\text{Ar}^*$ ($\times 10^{-7}$ s.c.c. g $^{-1}$)	$^{40}\text{Ar}^*/^{40}\text{Ar}_T$ (%)	Age (m.y.)	Laboratory
Faulting in Tarosero area								
Faulted rocks								
Olivine basalt	309	Tarosero	a	0.520	0.498	4.2	2.40 ± 0.62	S
Sodic trachyte	305	Tarosero	a	4.36	3.928	35.2	2.25 ± 0.06	S
Sodic trachyte	306	Tarosero	a	4.08	3.590	35.4	2.20 ± 0.06	S
Sodic trachyte	510	Tarosero	a	3.82	3.367	22.2	2.21 ± 0.10	S
Unfaulted rocks								
Sodic trachyte	490	Tarosero	b	3.97	{ 3.433 *	34.1	2.16 ± 0.06	S
					{ 3.236	71.2	2.04 ± 0.04	S
					{ 3.133	75.8	1.98 ± 0.03	S
Sodic trachyte	488	Tarosero	b	3.86	{ 2.960	50.8	1.90 ± 0.04	S
Main phase of rift valley faulting								
Faulted rocks								
Olivine basalt	304	Matunginini	d	0.686	0.549	4.4	2.00 ± 0.50	S
Olivine basalt	191	Ardai	c	2.85	{ 1.902	13.6	1.67 ± 0.08	N
					{ 2.011	22.7	1.77 ± 0.11	N
Olivine trachybasalt	139	Narabala	e	2.86	{ 1.418	41.4	1.24 ± 0.03	S
					{ 1.367	29.6	1.20 ± 0.04	S
					{ 1.343	25.1	1.18 ± 0.05	S
Unfaulted rocks								
Olivine trachybasalt	137	Narabala	e	3.34	{ 1.616	25.5	1.21 ± 0.05	S
					{ 1.533	30.8	1.15 ± 0.04	S
				3.53	{ 1.533	19.9	1.09 ± 0.05	N
					{ 1.643	31.2	1.17 ± 0.04	N
Olivine trachybasalt	11	Engaruka	g	3.03	{ 1.390	25.4	1.15 ± 0.04	N
					{ 1.342	18.4	1.11 ± 0.06	N
Olivine basalt	181	Kitete	f	0.78	{ 0.351	6.7	1.13 ± 0.16	N
					{ 0.381	10.6	1.23 ± 0.05	N
Olivine basalt	180	Kitete	f	2.42	{ 0.908	12.5	0.95 ± 0.08	N
					{ 1.035	21.1	1.08 ± 0.05	N
Minor phase of faulting on floor of rift depression								
Phlogopite	3	Kisetey	h	7.51	{ 1.71	4.1	0.57 ± 0.15	S
					{ 0.42	1.0	0.14 ± 0.15	S
Phlogopite	891	Loolmurwak	i	7.66	{ 1.63	5.6	0.53 ± 0.10	S
					{ 1.25	4.3	0.41 ± 0.10	S
Olivine nephelinite	105	Loolmurwak	i	1.36	{ 0.59	2.7	0.19 ± 0.08	S
					{ 0.08	1.4	0.14 ± 0.12	S

S, Determined at SURRC; N, determined at University of Newcastle.

$\lambda_e = 0.584 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_p = 4.72 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K} = 1.22 \times 10^{-4} \text{ g per g K}$; $^{40}\text{Ar}^*$ = radiogenic ^{40}Ar ; $^{40}\text{Ar}_T$ = total ^{40}Ar ; errors computed from $[E_K^2 + (1 + A/R)^2 E_{40}^2 + E_{38}^2 + (A/R)^2 E_{36}^2]^{1/2}$ where $R = (100 - A) = ^{40}\text{Ar}^*/^{40}\text{Ar}_T$ with error in K, $E_K = 1.5\%$ and errors in peak heights, $E_{40} = E_{38} = 0.5\%$; $E_{36} = 1\%$.

* Analysis of powdered sample.

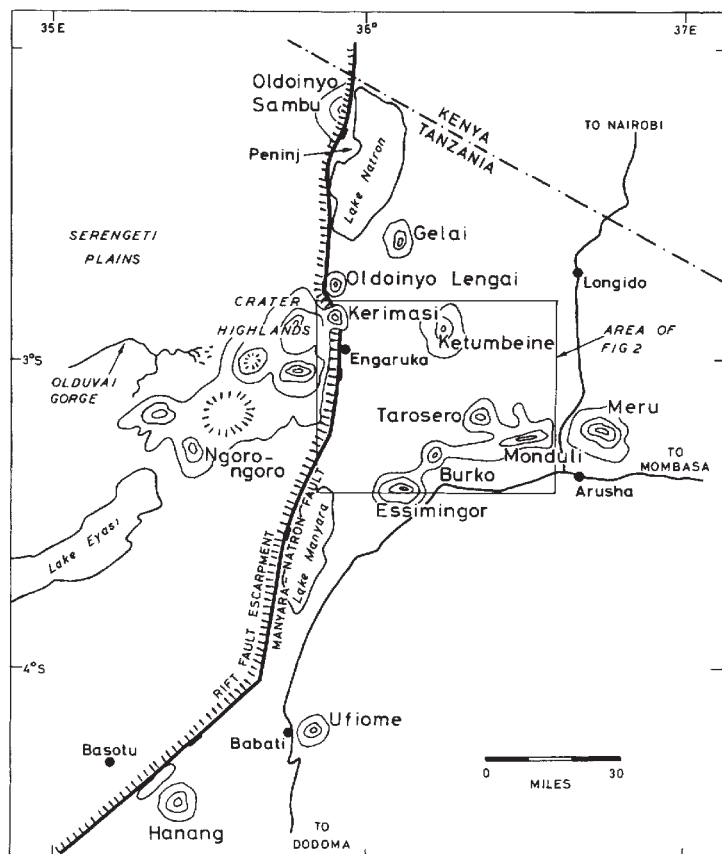


Fig. 1 Map of part of northern Tanzania, showing localities referred to in the text.

the western shores of Lake Natron, the Peninj Group sediments were deposited in a shallow fault-bounded basin that was in existence before the main faulting that has by now exposed these sediments. An early lava near the base of the Peninj sediments has been dated at 2.02 m.y., and olivine basalts higher in the succession (but pre-dating the main faulting) give dates of 1.4 to 1.6 m.y. (ref. 9). An additional minor phase of faulting that post-dates the main faulting has been recognised on the floor of the Rift depression between Engaruka and Lake Natron¹⁰. These later faults are tightly spaced and of small displacement, and may be analogous to the grid-faulting in the Magadi area just over the Kenya border. Small tuff-rings and cones of olivine-biotite-pyroxene tuffs erupted along these minor faults. The geological evidence shows that eruption occurred after the formation of the Kerimasi volcano but before the initial eruptions of the active carbonatite volcano Oldoinyo Lengai¹⁰.

To determine the age of the various phases of faulting, potassium-argon age determinations have been carried out on three groups of rocks carefully selected for their freshness from a number of critical areas. The rocks were crushed and sieved to +20 mesh and the radiogenic ⁴⁰Ar determinations made by isotope dilution on samples weighing several grams, using highly enriched ³⁸Ar as tracer. Argon isotope ratios were measured statistically using an MS10 mass spectrometer (Scottish Universities Research and Reactor Centre analyses) or an omegatron (University of Newcastle analyses). Potassium was determined by flame photometry on powdered fractions. Previous comparative analyses have shown that no systematic differences exist between measurements carried out in the two laboratories.

The locations of the rocks which were selected for investigation are given in Fig. 2 and the analytical results are presented in Table 1. It is convenient to discuss these results from the three groups of rocks separately and relate them, in turn, to the various phases of faulting.

(1) On the lower northern slopes of the Tarosero volcano (location (a) in Fig. 2) faulted olivine basalts and sodic trachytes are exposed in well eroded escarpments resulting from faults of small displacement. In the summit area of the volcano (b) these faulted rocks are buried by later extrusions of sodic trachyte. The well eroded nature of the fault-scarps suggests a relatively old age, possibly correlatable with the early movements on the Manyara-Natron Fault. This particular phase of faulting has been bracketed by dating the faulted rocks and later extrusives. The mean ages of the faulted and unfaulted rocks is 2.2 m.y. and 2.0 m.y. respectively, indicating that faulting took place in this area around 2.1 m.y. The ages are similar to those previously reported² for the nearby Ketumbeine and Monduli volcanic centres and may closely approximate the beginning of volcanic activity in the area. As the age of faulting is quite compatible with the age for the minor faulting that initiated the formation of the Peninj Basin, it probably also represents the first phase of movement along the Rift escarpment.

(2) Faulted basaltic rocks were collected from the top of younger steep escarpments resulting from the major phase of faulting at localities on the Ardai Plains, west of Arusha (c), the Matunginini escarpment (d) and at Narabala, on a major splay-fault, which diverges from the Manyara-Natron Fault, south of Engaruka (e). The ages measured for these rocks, which range from 2.0 m.y. to 1.2 m.y., are maxima for the age of the major phase of faulting.

At Narabala (e) and Kitete (f), basaltic rocks have been extruded from fractures resulting from the main faulting and form isolated flows at the base of the fault scarps. In addition, north of Engaruka (g) a flow of trachybasalt is interbedded with scree at the foot of the rift escarpment. Age measurements were made on these rocks to establish a minimum age for the major faulting. The ages are mainly around 1.1 m.y. and range from 1.2 m.y. to 0.95 m.y. At Narabala a particularly tight bracket has been found, as the mean of four measurements on two samples of unfaulted lava is 1.15 m.y., and the mean of three determinations on an earlier faulted flow is 1.21 m.y. The combined data indicate that the major phase of rift valley faulting took place between 1.15 and 1.2 m.y. This conclusion agrees well with the evidence that the major Kirikiti-Lengitoto fault (a

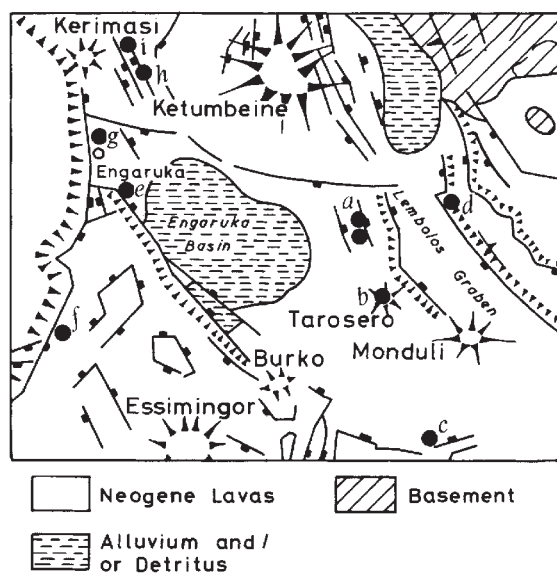


Fig. 2 Map showing localities of samples dated in this work. Localities: a, Lower slopes of Tarosero volcano; b, summit area of Tarosero volcano; c, Ardai Plains; d, Matunginini escarpment; e, Narabala; f, Kitete; g, Engaruka; h, Kisetey Hill; i, Loolmurwak crater.

major en echelon extension of the Manyara-Natron fault in South Kenya) developed in the period 1.3 to 0.9 m.y. (ref. 5). In addition, the major reversal of drainage and tectonic break between beds II and III of the Olduvai succession, both of which must be the result of some major Earth movement, are dated as post 1.5 m.y. and possibly at 1.0 m.y. (R. L. Hay, personal communication).

(3) The age of the later faulting on the floor of the Rift depression was examined by analysing phlogopite crystals from the tuff-rings at Kisetey Hill (*h*) and Loolmurwak Crater (*i*), as the geological evidence indicates that the faulting and eruption of these two minor volcanic features were probably contemporaneous. A sample of lava from a small lobate flow which has breached the tuff-ring at Loolmurwak was also analysed.

Although the measurements are characterised by a high analytical uncertainty on account of the small concentrations of radiogenic ^{40}Ar relative to atmospheric argon and other gases (2.14% CO_2 by weight in the lava) in the samples, the comparative youthfulness of this volcanic activity is confirmed. The calculated ages range from 140,000 to 570,000 yr and the average age of both phlogopites is around 350,000 yr. The best estimate for the age of eruption which can be derived from the data is about 370,000 yr and the faulting probably occurred near or shortly before this time. This faulting may be of similar age to the grid-faulting of the Rift floor in the Magadi area of Kenya, which has been bracketed between 0.8 and 0.4 m.y. using obsidian dating and pumice and fossil evidence⁵. As the late minor faulting and the main episode of Rift faulting bracket the eruption of the Kerimasi carbonatitic volcano¹⁰, this occurred within the period 1.1 to 0.4 m.y. The age of the minor faulting also places a maximum date of about 370,000 yr for the onset of eruptive activity at Oldoinyo Lengai.

The specimens were collected while one of us (J. B. D.) was a member of the Tanzanian Geological Survey. We thank the Natural Environment Research Council, the University of Newcastle, the University of Strathclyde and other Scottish Universities for financial support during the project, and Mr T. McMenamin for analytical assistance.

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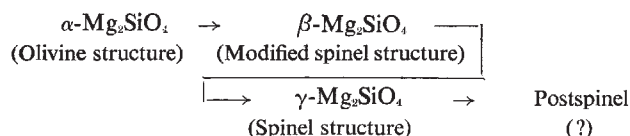
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Postspinel Phase of Forsterite and Evolution of the Earth's Mantle

THIS is the first announcement of success in the experimental confirmation of the postspinel phase of forsterite at high pressure and high temperature. Forsterite (Mg_2SiO_4) decomposes to periclase (MgO) and stishovite (SiO_2) at 330 kbar and 1,000° C. Forsterite is a major constituent of the Earth's mantle and the phase of forsterite composition at high pressure places a strong constraint on the nature of the transition layer and also on the composition and evolution of the Earth.

The profile of seismic wave velocity in the Earth's mantle shows two distinct stepwise increases at the depths of 400 and 700 $\text{km}^{1,2}$. These steps are thought to be due to the pressure-induced change of phase of forsterite³, since the Earth's mantle corresponds mostly to forsterite in composition. Thus extensive experimental trials to the phase change of forsterite and similar compounds have been made. The experimental results with forsterite⁴⁻⁸ are summarised as follows,



in the order of increasing pressure (depth). The transition

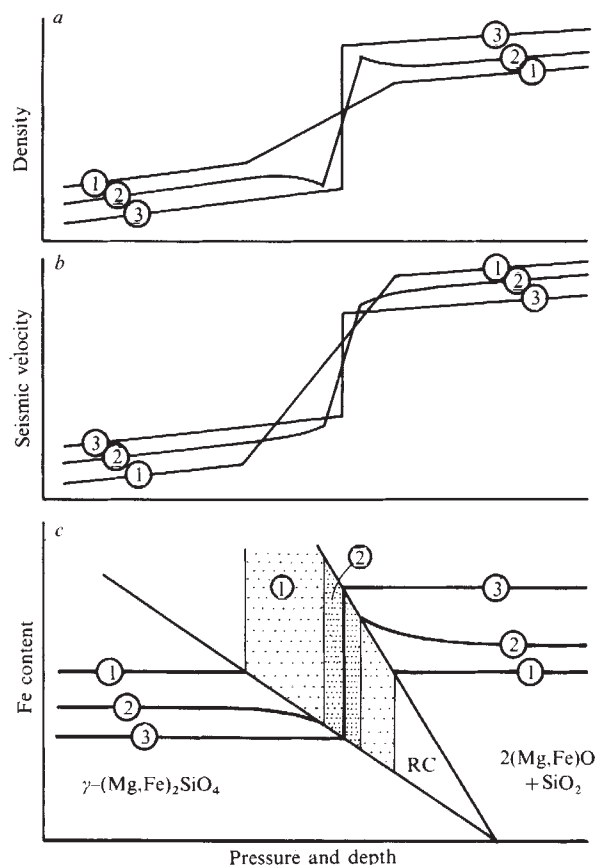


Fig. 1 Schematic drawing of the expected nature of the seismological step at a depth of 700 km. *a*, *b*, Density and seismic velocity profiles. *c*, Phase diagram of forsterite with some fraction of fayalite, and the profile of Fe concentration with depth in the mantle. RC, Region of coexistent phase. 1, Dynamic equilibrium or uniform concentration of Fe throughout the depth range; 3, static equilibrium between the upper and the lower mantles; 2, transitional state between 1 and 3. A sequence 1→2→3 may represent the change of structure of the transition layer at a depth of 700 km.

from olivine to spinel phases (β and γ) is correlated to the seismological step at a depth of 400 km as suggested by Bernal⁹. The seismological step at a depth of 700 km may be due to the transition from the spinel phase (γ -olivine) to a 'postspinel' phase, whose nature is not yet known.

From the physical viewpoint, Birch³ predicted that the transition would be to dense phases with a closely packed structure. Shimazu and Kumazawa¹⁰ may have been the first to suggest the decomposition to the mixture of MgO and SiO₂. Stishov¹¹ has shown clearly the possibility of decomposition to periclase (MgO) and SiO₂ with a rutile type structure, which Stishov and Popova¹² first synthesised at high pressure and which was later named 'stishovite'.

Many different possibilities have later been suggested on the basis of the accumulating experimental data on similar and/or related compounds; for example, transition to Mg₂SiO₄ with a strontium plumbate or K₂NiF₄ structure¹³, or transition to periclase plus MgSiO₃ with an ilmenite structure¹⁴. No direct experimental confirmation has been made, however, because a method of producing static pressures as high as 300 kbar combined with high temperatures has not been available.

We have been developing an apparatus using the MASS (Multiple-Anvil Sliding System) mechanism¹⁵, a new principle recently devised¹⁶. By using the apparatus under development, the high pressures up to 500 kbar in a volume of 0.005 cm³ are successfully reached (Masaki *et al.*, in preparation). The pressure produced is calibrated at room temperature on the basis of resistance measurements of calibrants on the pressure-raising cycle (Bi I-II=26 kbar; Bi III-V=76 kbar; Pb I-II=130 kbar; GaAs semiconductor-metal=190 kbar; ZnS semiconductor-metal=190 kbar; CdS resistance maximum=330 kbar, and ZnS resistance maximum=430 kbar)¹⁷. Pressures up to 350 kbar are produced in a pressure medium of 0.02 cm³, in which a carbon heater and Pt-Pt/13% Rh thermocouple are installed with a sample of 0.2 mg. Temperature is determined by measurements of EMF on the thermocouple without correction for the pressure effect and the surface temperature of the anvils, to which the thermocouple leads are connected. The starting material was a powder of synthetic forsterite (α -olivine), and the product run at 330 kbar and 1,000°C for 30 min was investigated by optical microscopy and X-ray diffraction. Table 1 shows that the product was certainly a mixture of periclase (MgO) (rock-salt structure) and stishovite (SiO₂) (rutile structure). No traces of γ -olivine or of unidentified material were detected.

Table 1 X-ray diffraction data of Mg₂SiO₄ run at 330 kbar and 1,000°C for 30 min (MoK α_1)

d_{obs} (Å)	d^* (Å)	Phase	$h k l$	I_{obs}
3.34	3.35	Carbon†	0 0 2	50
2.960	2.959	Stishovite	1 1 0	65
2.430	2.431	Periclase	1 1 1	20
2.242	2.246	Stishovite	1 0 1	20
2.103	2.106	Periclase	2 0 0	100
—	2.09	Stishovite	2 0 0	—
2.046	2.060	Diamond†	1 1 1	30
1.983	1.981	Stishovite	1 1 1	25
1.867	1.870	Stishovite	2 1 0	15
1.528	1.530	Stishovite	2 1 1	25
1.488	1.489	Periclase	2 2 0	30
1.480	1.478	Stishovite	2 2 0	20
1.338	1.333	Stishovite	0 0 2	10
—	1.322	Stishovite	3 1 0	—
—	1.291	Stishovite	2 2 1	—
—	1.270	Periclase	3 1 1	—
1.264	1.261	Diamond†	2 2 0	10
1.236	1.235	Stishovite	3 0 1	15

* ASTM Index Card.

† Carbon and diamond: contamination from carbon heater.

A pressure of 330 kbar corresponds to the pressure at a depth of 1,000 km in the Earth's mantle and the transition from the spinel phase to the oxide mixture is correlated to the

seismological step at a depth of 700 km, since there is no significant seismological step except a very small one at about 900 km depth in this range. No further phase change in the major constituent minerals is likely to take place in the mantle. Our results therefore suggest that the phase composition in the lower mantle is mainly stishovite and periclase. Of course, periclase should be a solid solution of MgO and FeO. Anderson¹⁸ has already shown that the seismological step at 700 km and the seismic wave velocity in the lower mantle are well explained by the assumption of postspinel phase having the same thermochemical and physical properties as mixture of (Mg,Fe)O and SiO₂.

The thermodynamic calculation shows that the decomposition of γ -olivine into a mixture of oxides is an endothermic reaction¹⁹. Therefore, the material will meet a large mechanical brake^{20,21}, when it is going to pass through the horizon of the transition at a depth of 700 km. This explains why the downgoing plate of lithosphere does not sink below 700 km and why earthquakes only occur above this level. In addition, the overall convective motion in the mantle, involving both the lower and the upper mantle, is hardly possible, not simply because of the high viscosity in the lower mantle²², but because of the mechanical stability originating from the heat and volume balance. This situation places a strong constraint on the evolution of the Earth. For example, if the time of the large scale geochemical differentiation in the Earth was realised through hydrodynamic migration of the material, it should be dated to a very early (unknown) stage of the Earth's history, unless diapirism or similar processes are proved to take place in the lower mantle.

As a straightforward consequence of the static contact of the lower and the upper mantles, there may be partition differentiation of Fe and Mg between the upper mantle (γ -olivine solid solution) and the lower mantle (periclase-wustite solid solution) through a horizon at 700 km. Since the equilibrium pressure of the phases containing Fe is lower than that of pure forsterite, the partition differentiation would result in an enrichment of Fe in the high pressure phase and a depletion in the low pressure phase. Figure 1 shows schematically the density and seismic velocity profiles of the mantle expected from the partition differentiation of Fe and Mg at a depth of 700 km. Even though the primitive mantle is uniform in Fe and Mg concentrations (1 in Fig. 1), the lapse of time will cause the transport of Fe to the lower mantle and that of Mg to the upper mantle (2 in Fig. 1). The migrations of Fe to the deeper part and of Mg to the shallower part in the lower mantle may be due to the diffusion in the gravity field and to the convective overturn of the material driven by the density reversal just beneath the 700 km horizon. In the upper mantle, there are many processes accounting for the mixing and migration of the material as inferred from such phenomena as the observed tectonic movement in the upper layers of the Earth and the expected low viscosity and high temperature gradient. The transition between olivine and spinel at a depth of 400 km is an especially strong factor promoting the migration of materials above and below this horizon, since this transition is unstable²³ due to the exothermic reaction when olivine transforms to spinel. After a sufficiently long time, the seismological step at 700 km would become a real first order discontinuity (3 in Fig. 1), which separates the iron-rich lower mantle and the magnesium-rich upper mantle. A notable characteristic of the transition at a depth of 700 km is that gravity differentiation and partition differentiation are completely cooperative. It is a stable interface associated with both a phase change and a chemical discontinuity.

Our interpretation is consistent with the difference between the equations of state on either side of the mantle inferred from the seismological data. When the velocity-density relation obtained by Press² is compared with the velocity-density-mean atomic weight systematics by Birch²⁴, for example, the lower mantle has an apparently higher mean atomic weight, suggesting higher concentration of Fe in the

lower mantle than in the upper mantle. It is noteworthy that no inflection is found in the velocity-density relation around a depth of 400 km. In addition, the Fe-rich lower mantle offers a reasonable explanation of the fact that the Fe/Mg ratio in the whole Earth calculated from the present composition of the upper mantle is low when compared with that calculated from chondritic and cosmic abundances. After the formation of the solid Earth, the Fe/Mg ratio in the upper layers of the Earth above 700 km horizon is expected to have been decreasing with geological time by the absorption of Fe into the lower mantle through the horizon at 700 km.

The sharpness of the seismological step at 700 km²⁵ may be partly due to the negative slope ($dT/dP < 0$) of the equilibrium temperature of γ -olivine and (Mg,Fe)O plus SiO₂. It is, however, better accounted for by the equilibrium between the Fe-rich lower mantle and the Mg-rich upper mantle. From our interpretation of the nature and evolution of the transition horizon at a depth of 700 km, the information concerning the sharpness of this seismological step is expected to be a good clue to the processes involved in the chemical differentiation, lateral inhomogeneity of the mantle, and so on. For example, Kanamori²⁶ has shown that the step at 700 km is unclear beneath the Japanese Islands, making a contrast to that beneath the North American continent²⁷. It is likely that the downgoing plate of lithosphere is disturbing the static equilibrium between the upper and the lower mantles.

The experimental details and the results on fayalite and olivine solid solution will soon be reported (Sawamoto *et al.*, in preparation).

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Possible superconductivity at room temperature

In this paper some observations are presented on an anomalous current in aluminum-carbon-aluminum (Al-C-Al) sandwiches, at room temperature, which in several respects behaves in the same way as the Josephson current might be expected to do. At first the switching effect was studied in Al-C-Al sandwiches discovered by Ovshinsky¹ and Pearson² in chalcogenide glasses and amorphous oxides. In carbon sandwiches subjected to proper electrical pulsing, changes in resistance of a factor of 1,000 were found, the changes being reversible and with a memory time of the order of a few days³.

When more experimental data were collected however, the effect seemed to be more complex. None of the known models proposed for switching phenomena in amorphous materials could be applied to this carbon system^{4,5}. Amorphous carbon is so different from materials used in switching devices that the switching in carbon may bear no relation to that in other materials. Most striking was the observation of a strong dependence of the current on a small external magnetic field.

The best results were obtained with samples produced in the following way: the first aluminium electrode was evapo-

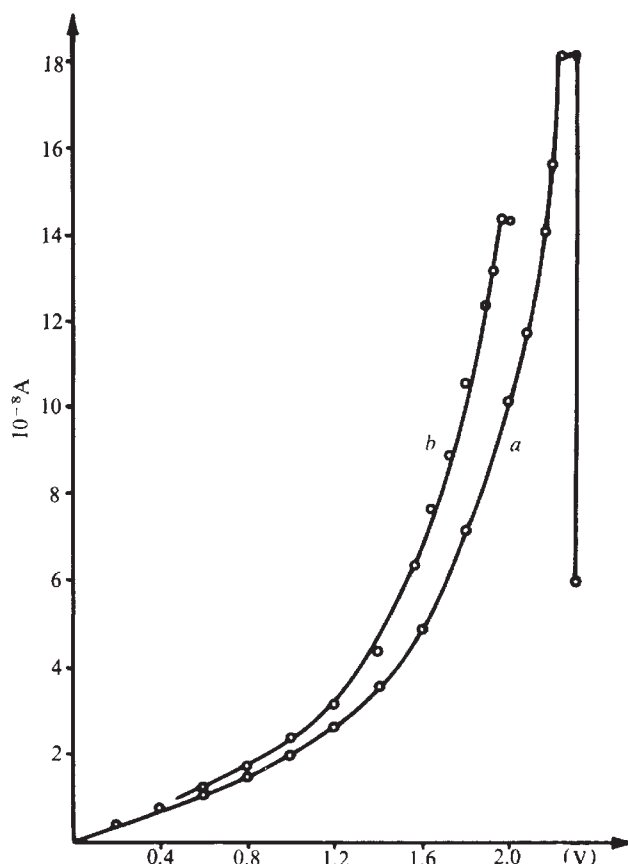


Fig. 1 Current-voltage characteristics for an Al-C-Al sandwich. a, Sudden decrease of current after passing $J_{\max}$; b, $J_{\max}$ approached by a slow increase of voltage.

rated on to a carefully cleaned porcelain substrate. Next the electrode was covered by polyfurfuryl alcohol (prepolymerised to a viscosity of about 10 cpoise and the sample heated up to a temperature of 620° C, the desired temperature being achieved in 80 min. At 300° C, the sample container was evacuated. Following this heat treatment, the upper electrode was deposited.

Current-voltage (I-V) curves were then taken. The current was measured by a mirror galvanometer. The voltage was increased in steps and after each increase of voltage a decrease of current with time was seen. This is probably due to the space charge (the original resistance of the sample is of the order of $10^8 \Omega$) because, after shorting the circuit, the current flows in the opposite direction like that in a charged condenser. The sample was maintained long enough at each voltage to reach equilibrium.

At about 2 V, a new phenomenon appeared: an increase of current with time at a constant or even decreasing voltage. This generation of conductivity continues for 1 to 2 h and the final current is two to five times larger than that at the beginning. The new state is quasi-stable and in good samples no essential decrease in conductivity can be detected for a few hours, but in a few days it decreases appreciably. Above a particular voltage, further increase in voltage gives rise to a decrease in current (curve *a* in Fig. 1), which is irreversible. To avoid this drastic change in conductivity, the initial voltage was increased gradually until the maximum current was attained (curve *b* in Fig. 1). Once $J_{\max}$ was reached, the voltage was lowered and the measurements were repeated in a new magnetic field. The magnetic field was produced by a coil, both the sample and the coil being inside a Mu-metal cover which shielded the sample from the magnetic field of the Earth.

Figure 2 shows the dependence of $J_{\max}$ on the applied magnetic field. When a field of 0.42 gauss is applied, the current is reduced by a factor of four to a minimum. At each successive integer multiple of 0.42 gauss, the current again goes through a minimum with successively decreasing maxima in between. First reduction of $J_{\max}$ was observed in various samples at fields as low as 0.1 gauss and as high as 1 gauss. While $J_{\max}$ varied by as much as two orders of magnitude.

Strong dependence on the magnetic field is known in tunnelling between two superconductors⁶. The Josephson DC tunnelling current in a magnetic field is given by the formula⁶:

$$J = J_0 [\sin(\pi\phi/\phi_0)/\pi\phi/\phi_0] \sin \delta$$

where ϕ is the flux enclosed by the area of the junction; ϕ_0 is the quantum unit of the magnetic flux; J_0 is a constant for a given junction at a constant temperature and δ is the phase difference which adjusts itself to the experimental conditions. As the current is increased, δ changes from zero for zero current to $\pi/2$ for the maximum allowable current. The expected dependence of $J_{\max}$ on the enclosed flux is

$$J_{\max} = J_0 [\sin(\pi\phi/\phi_0)/\pi\phi/\phi_0]$$

The Josephson current through a single junction is a periodic function of the flux enclosed in the junction. Such a dependence is the most convincing evidence that the current is a tunnelling current between two superconductors.

It is well established that in switching devices the current flows through a narrow path. The existence of a conducting path in carbon sandwiches can easily be seen when samples are destroyed because of overloading. In such samples, craters or pits in the aluminium electrodes are seen and are attributed to filament path formation. There are indications that in carbon sandwiches these paths are formed by a trail of conducting drops, manifesting themselves when the decay of the conducting state is observed as a function of time. The conductivity does not decay continuously but remains

approximately constant for a long time then drops rapidly to a lower value, remains roughly constant then drops again and so on. This seems to be a result of the inhomogeneous structure of amorphous carbon.

As far as is known, amorphous carbon consists of small crystallites of graphite-like layers with little layer stacking order and with strong lattice distortion. Studies of small-angle X-ray scattering from glassy carbon show that the impenetrable pore volume is made up of many submicroscopic

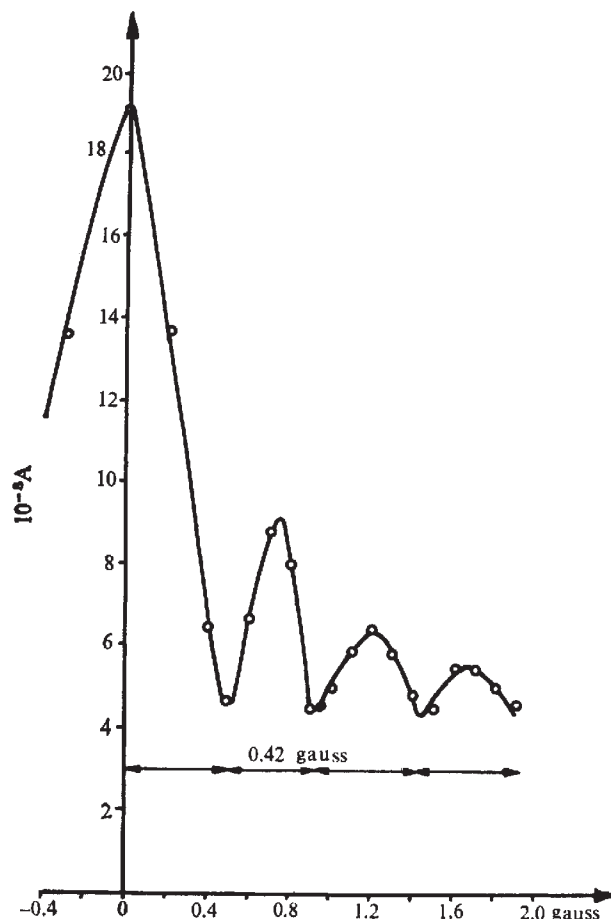


Fig. 2 Dependence of $J_{\max}$ on the applied magnetic field.

voids of average pore size about 25 Å. One could suppose that the tunnelling of electrons occurs through those voids but the area corresponding to the periodicity $\Delta H = 0.42$ gauss in the interference pattern is about $5 \times 10^{-7} \text{ cm}^2$. This is very much larger than the cross-sectional area of voids. On the other hand, the high percentage of samples which show the desired effect (about 30% when properly produced) indicates the existence of a natural mechanism producing barriers. One of the possibilities is the cracking of the carbon layer caused by the difference in the coefficients of the thermal expansion of the substrate and of carbon. The under part of the carbon layer adheres tightly to the porcelain, the upper part is free. It may be expected, therefore, that after heat treatment, cracks will always appear inside the carbon layer and that these cracks are responsible for the appearance of barriers.

A common feature of all the samples investigated is a long relaxation time; the time needed to determine $J_{\max}$ at a given magnetic field is about 0.5 h. Such long time constants

are known to appear when deep electron traps are involved and in amorphous carbon a high concentration of such traps is found.

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energy and obtained values three to ten times greater than the theoretical value and so suggested that plastic deformation was taking place at the roots of cracks. Sharp and Burnay⁵, on the other hand, assumed a value for surface energy and calculated the critical crack lengths to be much smaller than the flaws they had observed. They therefore proposed that fractures were initiated at cracks in the walls of the voids. Coyle *et al.*⁷ have also suggested that graphitic regions associated with impurities lead to reduced carbon fibre strengths. Failure mechanisms not involving impurities have also been put forward. For example, Le Maistre and Diefendorf⁸ proposed a circumferential arrangement of the layer planes and suggested that circumferential cracks are formed on cooling after the final heat-treatment due to thermal expansion anisotropy. Cooper and Mayer⁹, however, thought that cracks were

Tensile Strengths of Carbon Fibres

THE strength of carbon fibres prepared from commercially available polyacrylonitrile (PAN) fibres depends on the heat treatment temperature (HTT) and normally decreases¹ from a maximum after a HTT of about 1,500° C. Moreton² found that after all heat treatment temperatures (1,000–3,000° C) the strength is dependent on the gauge length indicative of a random distribution of flaws. Johnson³ and Thorne⁴ demonstrated that both surface and internal flaws are present, the latter due to foreign particles in the PAN precursor fibre reacting with the fibre during high temperature processing. Sharp and Burnay⁵ subsequently used high voltage electron microscopy to examine carbon fibres directly and concluded that, when the HTT is raised above about 1,800° C, inclusions react and volatilise to form voids. This results in the formation of misoriented three-dimensional graphite on the walls of the voids and decreases the strengths of the fibres.

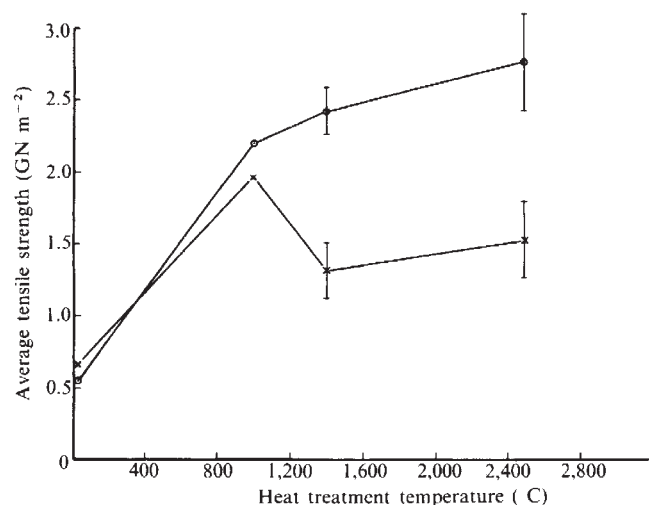


Fig. 1 Effect of heat treatment temperature on fibre strength. Error bars denote 95% confidence limits. ○, Clean room fibre; ×, control.

Attempts have been made to apply the Griffith brittle fracture criterion to carbon fibre strengths. Whitney and Kimmel⁶ used maximum flaw dimensions to calculate values for the surface

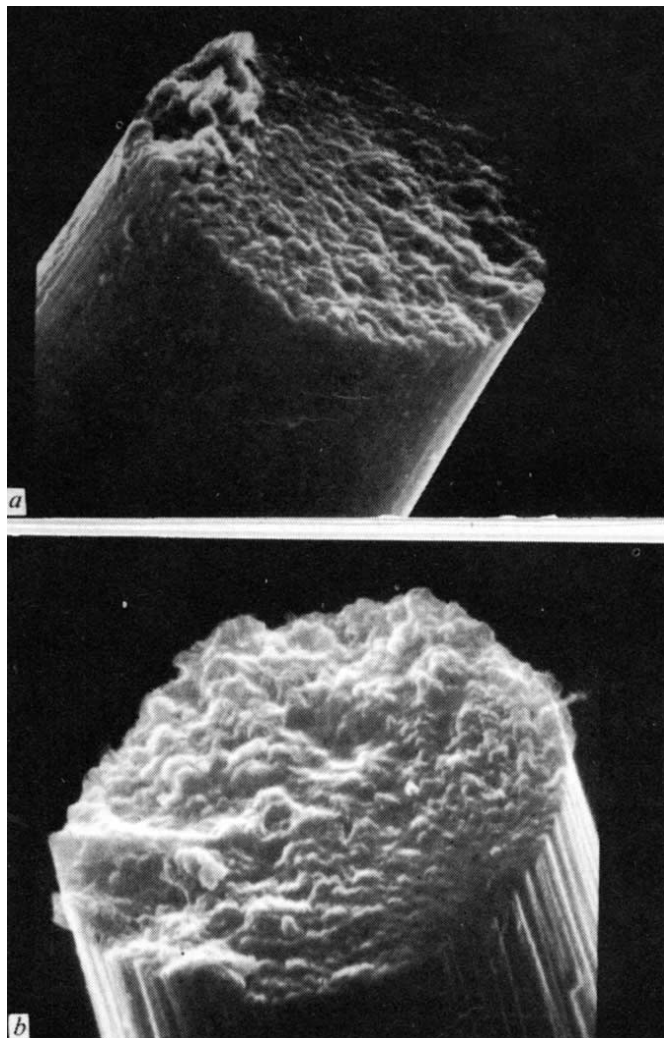


Fig. 2 Fracture surfaces of carbon fibres heat treated to 1,400° C. a, Control fibre with diameter 7.5 μm and strength 1.5 GN m⁻²; b, clean room fibre with diameter 8.1 μm and strength 2.6 GN m⁻².

formed as a result of dislocation pile-up with strength being inversely proportional to crystallite size¹.

In this laboratory it was decided that the effects of impurities must be removed if theories concerning the intrinsic strengths of carbon fibres were to be properly evaluated. Accordingly we have spun PAN fibres under class 100 clean room conditions (US Federal Standard 209A) (<100 particles per foot³ of air at 0.5 μm and above) from a solution of the polymer

filtered through a 1.5 μm filter. The fibres were then processed under clean conditions into carbon fibres using methods already described¹⁰ and heat-treated to 1,000, 1,400 and 2,500° C. Control samples were also obtained by spinning under normal laboratory conditions (approximately 10^6 particles per foot³ of air at 0.5 μm and above) a similarly filtered solution of PAN and these were converted to carbon fibre in the same way as the clean PAN fibres. Figure 1 shows the effect of HTT on the average strengths of the two batches of carbon fibre, each point representing an average value obtained from twenty tests using 5 cm gauge lengths. The strength of carbon fibres from the clean room precursor continued to increase with HTT whereas the control fibres suffered a marked strength decrease after the treatment to 1,400° C. Thus, after the final heat-treatment to 2,500° C the clean sample had a strength 80% higher than that of the control fibres. A further point of interest is that the strengths of the clean fibres were unaffected by gauge length over the range 1 to 5 cm whereas the control fibres had a marked gauge length effect, increasing by 45% on reducing the length from 5 to 1 cm. The Youngs moduli of the clean fibres and the controls were virtually the same after each heat treatment but this had been expected as both batches had been spun and processed in the same way. Scanning electron microscopy of the fracture surfaces of fibres heat treated to 1,400 and 2,500° C showed a complete absence of the recognisable internal flaws found in commercial carbon fibres and in all cases failure seemed to have started at the surface. The surface flaws in the control samples were more obvious than those in the clean fibre and an example of each is given in Fig. 2.

The results reported here emphasise the importance of removing impurities from the PAN precursor fibre and show that the decrease in strength with HTT previously found is not an inherent characteristic of carbon fibre. Recently Reynolds and Sharp¹¹ have put forward a failure mechanism based on shear taking place in misorientated crystallites and their model does predict strength to increase with HTT. Finally, it must be added that further increases in strength should be possible if the small flaws still present in the fibres could be removed. Not too much importance should be attached to the actual strength values of the carbon fibres quoted in this investigation but only trends should be considered. A total of 0.5 g was used. Much larger quantities give higher strengths, presumably due to some surface healing by carbon deposition from pyrolysis gases, as is well known. But it now seems obvious that the strength values of carbon fibres are a direct consequence of the external and internal cleanness of the initial PAN fibres.

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Precambrian Microfossils of Carpentarian Age from Bungle Bungle Dolomite of Western Australia

I WISH to present some preliminary results and interpretations of the examination of a well preserved assemblage of mid-Precambrian microfossils. This previously unknown microflora of blue-green and green or red algal affinities is from chertified stromatolitic dolomites of the Bungle Bungle Dolomite which outcrops in the Osmond Range of Western Australia. There are no published radiometric ages for this formation but the Mount John Shale Member of the overlying Wade Creek Sandstone has been dated (Rb/Sr method) at $1.128 \pm 0.1 \times 10^9$ yr BP¹. Regional studies and the angular unconformity between the Bungle Bungle Dolomite and Wade Creek Sandstone indicate that the former is considerably older than this minimum and that an age of about 1.5×10^9 yr for the microbiota described here is consistent with the data available. The Bungle Bungle Dolomite consists of at least 1.3 km of dolomite, dolomitic shales, shales and sandstones. Well preserved stromatolites occur at certain levels and have been briefly described elsewhere¹. The samples used in this investigation are from the measured section in the upper part of the formation at 17° 13' S 128° 23' E.

The microfossils are preserved in dark brown to black, fine grained cherts. Organic matter, either in a finely divided, unstructured form or as structurally preserved microfossils is responsible for the dark colour of the chert. In petrographic thin section (30 μm thick) the organic matter is pale amber to dark brown with the microfossils being generally darker than the intimately associated unstructured material. The chertified horizons in the Bungle Bungle Dolomite have a complex diagenetic history the elucidation of which is further complicated by the effects of tropical weathering. Dolomite and silica minerals are mutually replacive^{2,3} making unequivocal interpretation of petrographic evidence difficult. In broad terms, field and petrographic data indicate that the fossiliferous chert is diagenetic in origin, often replacing the original carbonate sediment.

In some of the microfossils, fine structural details are preserved, while others are modified or destroyed by diagenetic and subsequent processes. Extreme variations in the state of preservation are often juxtaposed and associated with subtle textural differences in the chert. Compaction and chemical alteration of much of the organic matter occurred before the initial chertification while the replacement of chert by very late dolomite is responsible for the destruction of much fossiliferous material.

Only a selection of the diverse Bungle Bungle microbiota is presented here and a more systematic account will be published elsewhere. A fairly restricted range of vegetative structure is exhibited by the coccoid forms in the assemblage. As a result of irregularities in fossilisation and subsequent processes, morphological characteristics are subject to great variation. This morphological inconsistency is superimposed upon natural variation making the delineation of discrete morphotypes, though possible, often difficult and very speculative.

The most common coccoid forms are unicells, dyads and tetrads of cells 1–5 μm in diameter (Figs 1a and b) many of which possess double or multilayered walls. This broad group, which includes at least four types, may occur as isolated cells, in clusters of up to a hundred cells or in close, almost colonial associations. The cells of another group with similar gross morphology contain inclusions, which may be in the form of dark spots about 1 μm across (Fig. 1c) or as apparently membrane-bound structures 2–3 μm in diameter containing minute dark granules (Fig. 1d). Isolated colonies of spherical to ellipsoidal cells 2–4 μm in diameter are not uncommon (Fig. 1e). Individual colonies may be up to 50 μm across, comprising several hundreds of cells, many of which contain

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minute (about 0.5 μm) inclusions, and whose shape may be modified by mutual compression. Some of these colonies are preserved in the process of disintegration into smaller colonies or into separate cells.

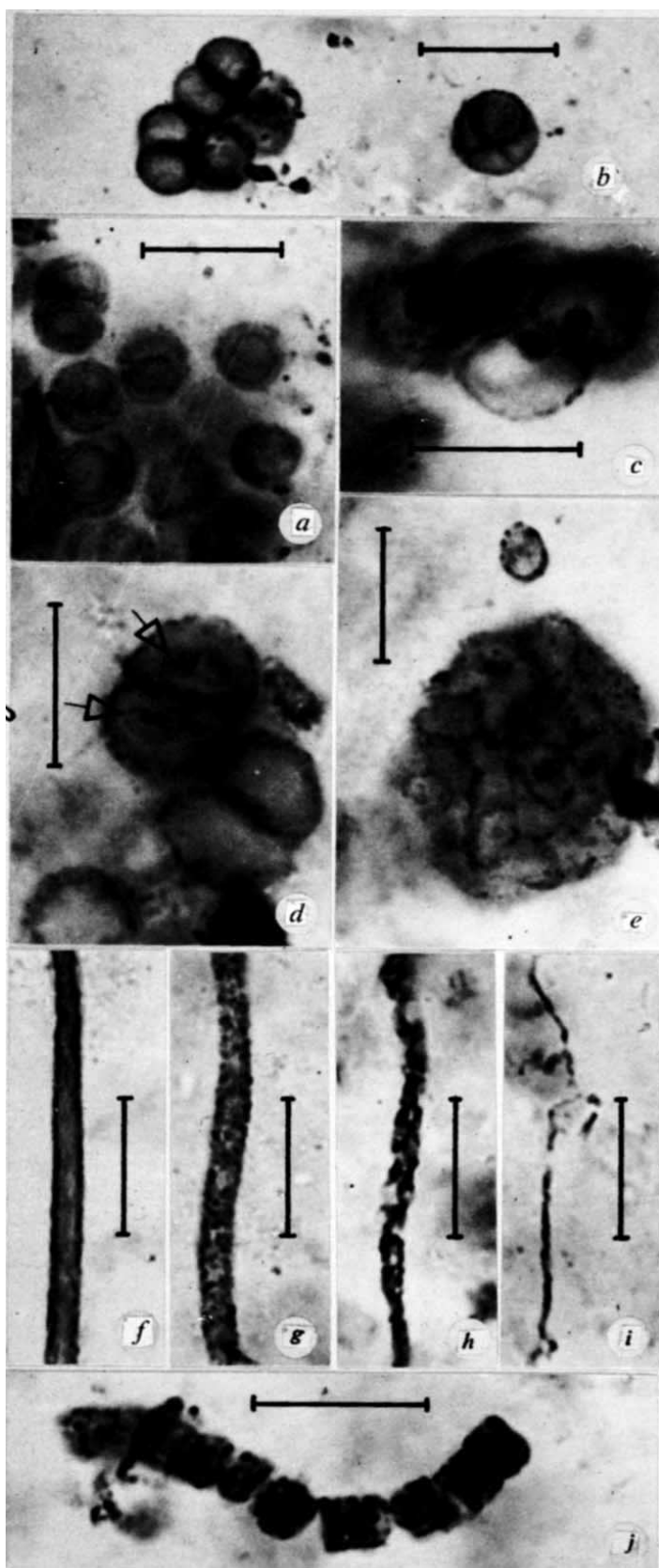


Fig. 1 Structurally preserved organic microfossils from the Bungle Bungle Dolomite. All micrographs are of petrographic thin sections of certified stromatolites in plane polarised light. All scale bars represent 10 μm .

Filamentous forms are less abundant and not as well preserved as the coccoid forms. The most common type of filament (Fig. 1f-h) is non-septate, unbranched, 1.5–2 μm in diameter and up to 500 μm long. Variations in preservation often give these filaments a septate appearance (Fig. 1g and h). Smaller filaments 0.6 μm or less in diameter occur and variously seem to be septate, non-septate, spirally twisted or composed of strings of rod-shaped cells (Fig. 1i). It is difficult to determine whether these are separate types or preservational artefacts, since their detailed morphological features are at or below the limit of effective light optical resolution. Septate filaments are rare and usually fragmentary with the constituent cells often nearly separate. The most frequent type (Fig. 1j) is composed of cylindrical cells 2.5–3.5 μm in diameter and 1.5–2.5 μm long with apices terminated by a small hemispherical cell.

The size, vegetative form, and morphology of most of the microbiota are comparable with living cyanophycean algae. Coccoid forms with layered walls resemble members of the Chroococcaceae, and the septate filaments look like trichomes of the Oscillatoriaceae. Living oscillatoriaceans possess the facility of gliding motility and the non-septate filaments may be abandoned sheaths of such algae. The specimens arrowed in Fig. 1d resemble plasmolysed cells. Since the protoplasm of living blue-green algae is highly viscous and difficult to plasmolyse⁴ it may be that, as suggested elsewhere⁵, such forms (Fig. 1c and d) represent eukaryotic algae (Chlorophyceae or Rhodophyceae) with their contents and organelles preserved.

Stromatolites are laminated organosedimentary structures that at the present day are formed in shallow water by associations of algae dominated by the Cyanophyceae. The striking similarity between detailed sedimentological features and the morphologies of the associated organisms in Recent^{6,7} Bungle Bungle, and other stromatolitic deposits⁵ supports the interpretation of the Bungle Bungle microbiota as a stromatolite-producing assemblage of coccoid and filamentous cyanophycean algae associated with less common coccoid eukaryotic algae.

In an evolutionary context the Bungle Bungle microbiota is comparable with other Precambrian stromatolitic assemblages, especially that from the Amelia Dolomite⁸, and is consistent with the evolutionary models proposed by Cloud⁹ and Schopf^{10,11}. It must be stressed, however, that stromatolitic microbiotas on which these models are largely based, are from the same restricted environment and hence, represent a strongly biased sample of Precambrian life. In the Bungle Bungle microbiota, however, elements from other environments are present and exemplified by the colonial forms (Fig. 1e) which are not members of the stromatolite-producing assemblage but invariably occur in sediment/detritus rich laminae. These allochthonous forms and microfossils described elsewhere^{12,13} from non-stromatolitic Precambrian sediments suggest that life had become considerably diverse by 1.5×10^9 yr BP and that much remains to be discovered about the ecological diversifications and evolution of early life.

Dr M. D. Muir (Imperial College, London) and Mr K. A. Plumb (BMR, Canberra) collected the samples described here in 1972. I acknowledge their help and encouragement during the work which was sponsored by a research assistantship from the Natural Environmental Research Council.

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Spheroidal particles produced by cavitation erosion

SMOOTH spherical particles have been observed during the microscopic analysis of wear surfaces (ref. 1 and R. S. Miller, personal communication) and of wear particles² using a new technique called ferrogram developed by West-

cavitation erosion apparatus and ASTM standard test procedures. Tests were conducted on annealed SAE 52100 steel (supplied by the Timken Company) in SAE 10w non-detergent (Quaker State) lubricating oil and on aluminium 1100-F in distilled water.

Analysis of the lubricating oil samples done at Trans-Sonics by S.W.D., A.P.T. and V.C.W. using the ferrographic technique revealed the existence of spheroidal particles. Fig. 1b shows optically perfect spheres produced by cavitation of SAE 52100 steel in SAE 10w oil. Similar analysis of the oil samples and of the aluminium erosion particles using techniques developed at the Institute of Ocean Science and Engineering of Catholic University also revealed such spherical particles (Fig. 1b). Using an electron probe micro-analyzer A.P.T. conducted X-ray analysis to determine the composition of these micro-spheres. Figure 1c and d show that the spheroids are indeed steel and aluminium.

This discovery that cavitation erosion can produce very smooth spheres has many practical and scientific implica-

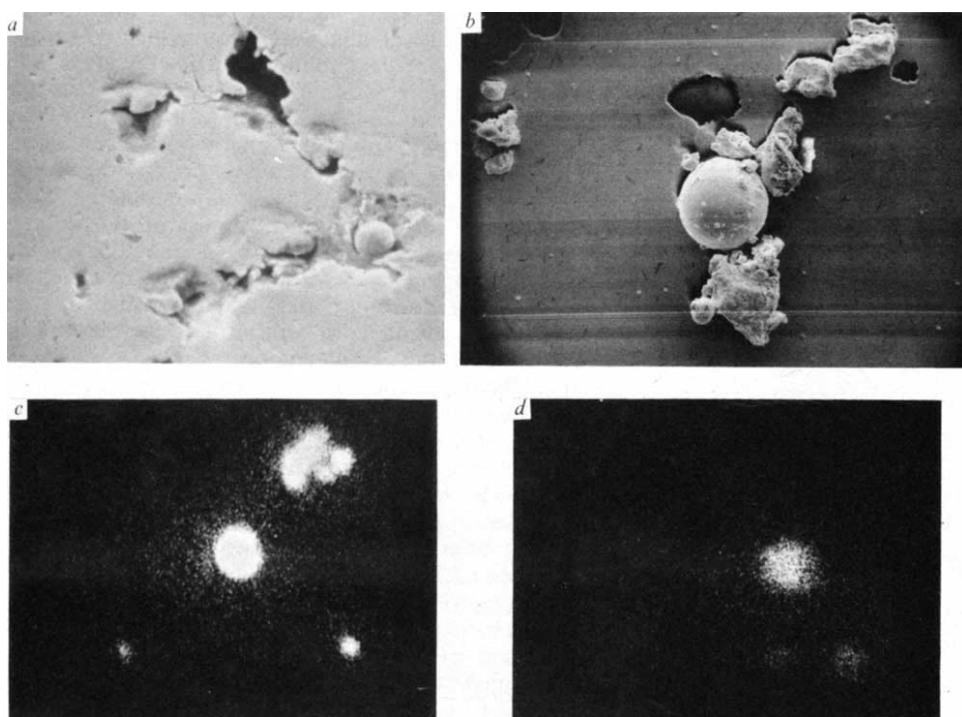


FIG. 1 a, Spherical particle observed in microcrack in an automotive ball bearing (R. S. Miller, personal communication); b, photograph of a smooth spherical particle observed in the oil cavitated for 40 h in an ASTM standard cavitation erosion apparatus (courtesy of Dr Ruff at the National Bureau of Standards); c, X-ray image scanned for aluminium in an electron probe microanalyser; d, X-ray image scanned for steel in an electron probe microanalyser.

cott². These investigators speculated that the spherical particles were produced by cavitation bubbles occurring at the contact surfaces of the bearing. The possibility of cavitation in bearings is well known³; however, it was difficult to understand how these bubbles could produce such perfect microspheres (in the range of 1 to 10 μm in diameter). To verify that cavitation erosion could indeed produce such spheres, we conducted careful experiments with the help of the American Society for Testing and Materials (ASTM) standard vibratory cavitation erosion apparatus; this communication reports the results of this investigation.

R. S. Miller (personal communication) noticed a spherical particle in one of the scanning electron micrographs (taken by Sperry Corporation) of an automotive ball bearing (Fig. 1a). About the same time, Scott and Mills¹ and Westcott² observed such spherical particles in bearings. Scott and Mills observed them on the fracture surfaces of a ball but Westcott used his new technique², called ferrographs, and observed them in various lubricating fluids after their use in bearings.

At the suggestion of Doroff and Miller, A.P.T. conducted controlled experiments using the ASTM standard vibratory

tions in identifying, understanding, and solving many erosion and wear problems. We are pursuing these investigations further.

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Nephrite (jade) Occurrence in the Great Serpentine Belt of New South Wales, Australia

THIS communication is to report the presence in the Great Serpentine Belt of New South Wales, Australia¹⁻³ of a nephrite (jade) occurrence. Within the Great Serpentine Belt that outcrops discontinuously for more than 350 km in northern New South Wales⁴ (Fig. 1) several economic min-

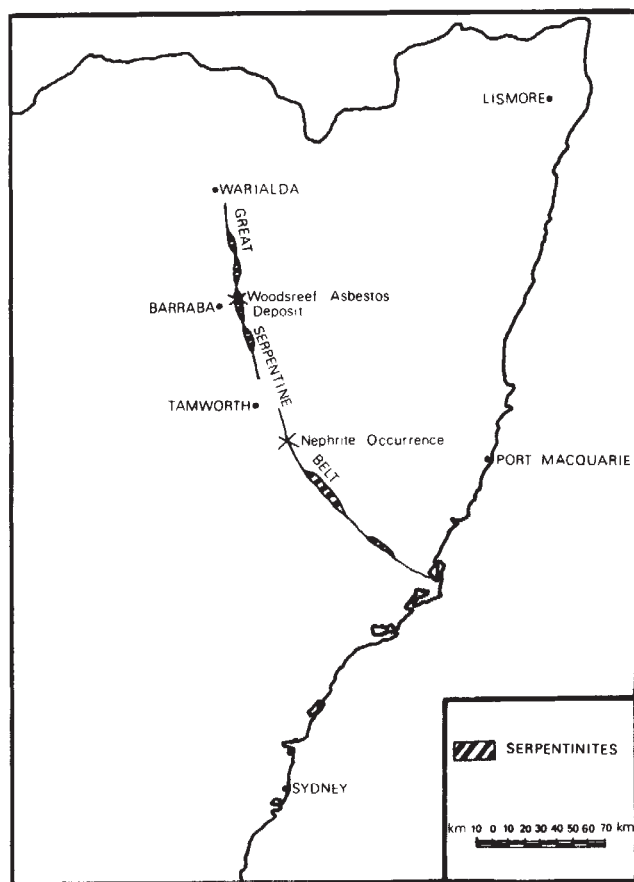


FIG. 1 Location of the Great Serpentine Belt in northern New South Wales, Australia.

eral occurrences have been discovered and developed in recent years. The Woodsreef asbestos deposit, near Barraba, the largest deposit of its type in the Southern Hemisphere, is by far the best known of the recent discoveries.

The lensoidally shaped nephrite occurrence 24 km south-east of Tamworth may prove to be Australia's first economic deposit as high quality gemstones have been cut from the occurrence. Geological investigations are currently in progress to determine the size, overall grade and economic potential of this very interesting mineral occurrence.

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BIOLOGICAL SCIENCES

Transamination is a Major Pathway of L-Dopa Metabolism following Peripheral Decarboxylase Inhibition

WHEN L-3,4-dihydroxyphenylalanine (L-dopa) is administered to human subjects¹, the greater proportion is metabolised by decarboxylation, a small amount by O-methylation and traces only by transamination². It seems likely that the benefit resulting from L-dopa therapy in Parkinsonian patients derives largely from the generation of one of its metabolites, dopamine, by decarboxylation within the central nervous system³. Less than 5% of administered L-dopa becomes available for this purpose: most of the dose is decarboxylated peripherally⁴. There is good reason to believe that the dopamine so formed is unable to penetrate the blood-brain barrier to any great extent⁵, so that little of the original dose is available to contribute to the therapeutic response. By blocking peripheral decarboxylase, however, certain decarboxylase inhibitors^{6,7}, which are themselves unable to cross the blood-brain barrier to any significant extent⁸, bring about an accumulation in the plasma of administered L-dopa, thus providing a higher concentration gradient to enter the central nervous system and allowing lower oral doses of L-dopa to be employed therapeutically⁹.

It has so far been assumed that, apart from some increase in the formation of 3-O-methyldopa¹⁰, peripheral degradation of L-dopa is largely prevented by the concomitant use of a decarboxylase blocking drug. We now present evidence that the combination of drugs results in a major diversion of L-dopa metabolism to a pathway where transamination substitutes for decarboxylation.

Thirteen patients with idiopathic Parkinsonism, taking part in a double blind comparison of L-dopa plus L- α -methyl-dopahydrazine (MK 486) against L-dopa alone¹¹, were investigated. Urine samples (24 h), preserved at -15°C with 6 N HCl (25 ml), were collected during the following treatment regimens: (1) before treatment (controls); (2) whilst receiving an optimal oral dose of L-dopa (1.0-5.75 g per 24 h; mean=3.5 g per 24 h) ('normal' dose experiment); and (3) whilst receiving a reduced dose of L-dopa (0.1-2.0 g per 24 h; mean=0.65 g per 24 h) plus oral MK 486 (300 mg per 24 h) ('low' dose L-dopa plus MK 486 experiment). Urine samples were also collected from (4) three of these patients during treatment with their own particular reduced dose of L-dopa (0.3-1.1 g per 24 h; mean 0.62 g per 24 h) employed during regime (3) but in the absence of MK 486 ('low' dose L-dopa experiment). Urinary *m*-hydroxyphenylacetic acid (*m*-HPAA), *p*-hydroxyphenylacetic acid (*p*-HPAA), homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were quantified as described previously^{12,13}, except that silylation was performed at 65°C for 1 h, using N,O-bis(trimethylsilyl)-acetamide (BSA, 0.2 ml) and dioxane (0.1 ml) containing *n*-eicosane ($8\text{ }\mu\text{g ml}^{-1}$) as internal standard. Urinary 4-hydroxy-3-methoxyphenyllactic acid (VLA), 4-hydroxy-3-methoxyphenylglycol (HMPG) and 4-hydroxy-3-

Table 1 Effect of MK 486 on urinary metabolites of L-dopa

Drug regimen	<i>m</i> -HPAA	<i>p</i> -HPAA	HVA	Metabolite DOPAC	VLA	HMPE	HMPG
Control experiment (<i>n</i> =13)	3.4 (0.52)	9.6 (0.85)	6.6 (0.60)	2.8 (0.34)	0.1	0.17 (0.01)	1.64 (0.12)
'Normal' dose experiment on L-dopa alone (<i>n</i> =13)	10.0 (1.73)	11.2 (1.36)	801 (169)	589 (132)	16.0 (4.9)	1.76 (0.37)	2.19 (0.36)
'Low' dose experiment on L-dopa alone (<i>n</i> =3)	5.6 (0.92)	13.3 (3.01)	142† (39.1)	91.3† (39.8)	2.6* (0.86)	0.39 (0.20)	1.55 (0.28)
'Low' dose experiment on L-dopa plus MK 486 (<i>n</i> =13)	5.7 (1.29)	13.5 (2.59)	52.5† (11.6)	17.0† (4.36)	96.5* (19.1)	0.30 (0.03)	1.74 (0.25)

m- and *p*-HPAA=*m*- and *p*-hydroxyphenylacetic acid; HVA=homovanillic acid; DOPAC=3,4-dihydroxyphenylacetic acid; VLA=4-hydroxy-3-methoxyphenylacetic acid; HMPE=4-hydroxy-3-methoxyphenylethanol; HMPG=4-hydroxy-3-methoxyphenylglycol.

Values are means $\pm$ s.e.m. (bracketed), expressed as mg per 24 h.

Significance of difference between respective urinary metabolites in patients on 'low' dose alone with those on 'low' dose plus MK 486.

*= $P < 0.05$. †= $P < 0.01$.

methoxyphenylethanol (HMPE) were assayed as before¹⁴, except that the derivatised extracts were finally reconstituted in ethyl acetate (0.3 ml) containing α -1,2,3,4,5,6-hexachlorocyclohexane (α -HCH, 1 μ g ml⁻¹) as internal standard.

The results are given in Table 1. In reduced dose experiments, the urinary excretion of *m*- and *p*-HPAA, HMPG and HMPE was relatively unchanged by MK 486. There was, however, a highly significant drop in output of the decarboxylation pathway metabolites, HVA and DOPAC, during MK 486 treatment, in agreement with the findings of others¹⁵. In accordance with a prediction by one of us², there was a large increase in output of the transamination metabolite, VLA. The extent of this shunting to an alternative degradation route is demonstrated in Fig. 1 where values are shown for three patients who were, respectively, receiving 300, 1,100 and 400 mg L-dopa per 24 h, with and without MK 486. A complete reversal in the ratios of decarboxylation to transamination series metabolites was observed, a useful index in itself, perhaps, of *in vivo* decarboxylase inhibition.

To what extent the increased output of VLA represents transamination of L-dopa itself followed by O-methylation rather than degradation of 3-O-methyldopa (Fig. 2) cannot be decided on the evidence so far available. The amount

of 3,4-dihydroxyphenylacetic acid present might have been expected to shed light on the problem. It was indeed sought but not detected in any sample, even though reference standards taken through the procedure were recovered from urine in high yield. This finding was first interpreted as indicating that the VLA derived solely from 3-O-methyldopa, of which it is known to be the major metabolite¹⁶. A study of the further metabolism of immediate keto-acid transamination

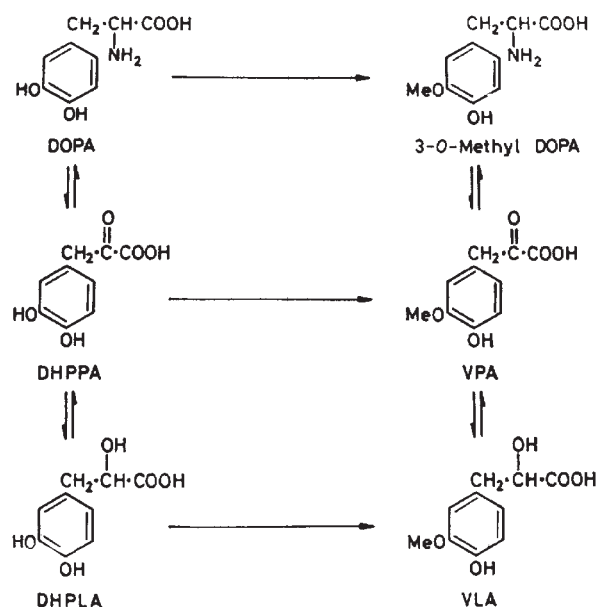


Fig. 2 Transamination pathways of L-dopa metabolism.

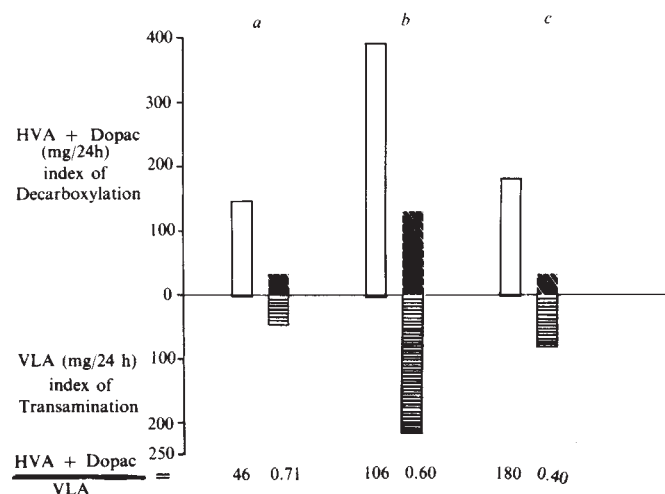


Fig. 1 24 h urinary output of 4-hydroxy-3-methoxyphenylacetic acid (vanillic acid, VLA) before (amounts too small to illustrate clearly) and after (horizontally shaded columns) and homovanillic acid (HVA) plus 3,4-dihydroxyphenylacetic acid (DOPAC) before (open columns) and after (diagonally striped columns) peripheral decarboxylase inhibition with MK 486 in three Parkinsonians taking oral L-dopa as follows: Patient a=300 mg, b=1,100 mg, c=400 mg per 24 h.

products in the rat (B. L. Goodwin, C. R. J. Ruthven and M. Sandler, in preparation) has, however, revealed the rather surprising finding that 4-hydroxy-3-methoxyphenylpyruvic acid is freely converted to the corresponding lactic acid whereas 3,4-dihydroxyphenylpyruvic acid does not undergo this type of reduction. The solution to this problem must await further study of the phenyllactate-forming enzyme^{17,18} and quantitative analysis of the pyruvic acids themselves, a procedure which has not so far been technically feasible because of the formation of multiple derivatives.

With high concentrations of L-dopa in the circulation protected from decarboxylation, there seems to be no very good reason why considerable amounts of 3,4-dihydroxyphenylpyruvic acid should not be generated. In terms of the therapeutic response, the role of any Schiff base which might

be derived from it¹⁹ is still speculative. As predicted¹², this keto-acid is a good substrate for *p*-hydroxyphenylpyruvate oxidase²⁰, which converts it to 2,4,5-(3,4,6-)trihydroxyphenylacetic acid. The trihydroxy acid has recently been identified unequivocally in the urine of Parkinsonian patients during L-dopa treatment (J. H. Fellman, personal communication). Its distribution of ring hydroxy groups in relation to the side chain is identical to that of 6-hydroxydopamine, the effects of which on the central or peripheral nervous system can be dire²¹ once it is taken up into catecholamine-binding sites. It is to be hoped that concentrating mechanisms do not exist for the trihydroxy acid which could well possess similar properties if present in high enough local concentration.

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Evidence from *Tfm/0* that Androgen is Inessential for Reproduction in Female Mice

THE testicular feminisation syndrome in affected XY male mice is the result of the action of the X-linked *Tfm* gene¹, which causes a failure of target organs to respond to androgen. The question arises whether genetic females affected with the gene would be reproductively abnormal in any way. Androgen is produced in the normal mouse ovary; its functional significance is not understood but it has been claimed that a response to androgen is essential for ovulation and mating². If so, then females homozygous or hemizygous for the *Tfm* gene (*Tfm/Tfm* or *Tfm/0*) might be unable to reproduce. Ohno *et al.*³, have recently described six *Tfm/0* female mice which collectively showed remarkably rapid ovarian degeneration, more rapid than in *+/0* mice, thus leading to the conclusion that a response to androgen is necessary for normal ovarian function in mice. The fertility of these mice had not, however, been tested. We here report results with two *Tfm/0* mice, both of which proved to be fertile and produced litters.

Both animals were born in an experiment in which female mice were irradiated with a dose of 100 rad X rays on the morning after conception, a treatment which yields a high frequency of XO offspring⁴. The parental crosses were *Tfm +/+ + Ta + ♀ × + + Gs/Y ♂*, and the two *Tfm/0* animals were the progeny of different pairs several months apart. Both were wild type in colour and externally looked female. They were distinguished from *Tfm/Y* by the fact that their vaginae opened at an age typical for normal females, that is, before 6 weeks, whereas in *Tfm/Y* the vagina typically does not open until about 3 months of age.

The two animals were mated at 46 and 39-d-old to a *Blo/Y* and a *Ta/Y* male respectively. Both proved fertile, the first having four pregnancies and the second five (Table 1). The offspring confirmed the presumed genotypes of the mothers: there were typical *Tfm/Y* offspring, confirming that both mothers carried the *Tfm* gene, and presumed XO offspring, indicating that both mothers were themselves XO. This latter point was also confirmed by chromosome counts, on tissue culture of ear skin for the first and on corneal epithelium for the second animal. In both cases these showed only thirty-nine chromosomes. Therefore, both mice were genetically proven to be *Tfm/0*.

Since these animals obviously ovulated and mated repeatedly, went through normal pregnancies, and reared offspring, it is clear that, in the mouse at least, a response to androgen is not essential for any of these functions.

The question arises whether the continued fertility up to 6 months of age of our two animals is in conflict with the

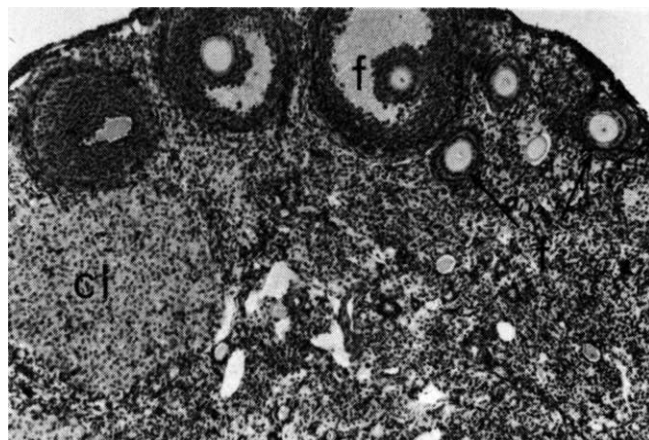


Fig. 1 Ovary of pregnant *Tfm/0* mouse, aged 6 months. Follicles at all stages (f) and corpora lutea (cl) present. $\times 72$.

finding³ of Ohno *et al.* of marked deterioration in *Tfm/0* ovaries as early as 8 weeks. The reproductive performance, in terms of litter size, number of litters, and reproductive lifespan of our animals was within the range found for XO mice⁵, but below average. Thus on the evidence of reproductive performance their ovaries might have been either more severely affected than +/0 ovaries, or comparable with them. Histologically, however, our findings did differ from those of Ohno *et al.* In serial sections the ovaries of both animals contained apparently viable oocytes and were no more severely affected than those of +/0 animals of comparable age. In the second animal, killed at 6 months of age, the only obvious abnormalities were a shortage of primordial oocytes and an excess of atretic follicles (Fig. 1). Otherwise the ovary was essentially normal, with follicles at all stages and a number of corpora lutea. Many of the antral follicles were degenerating, but this animal was pregnant when killed and degeneration of large follicles is characteristic of pregnancy⁶.

Table 1 Breeding tests of two *Tfm/0* mice

Animal	Age (d)	No. born	No. weaned	Offspring		
1 Mated	46			<i>Bl</i> /+	<i>Bl</i> /0	<i>Tfm</i> /Y
Litter 1	70	3	3	3	—	—
2	90	5	5	3	—	2
3	132	2	2	2	—	—
4	175	2	2	1	1	—
Killed	277					
				<i>Ta</i> /+	<i>Ta</i> /0	<i>Tfm</i> /Y
2 Mated	39					
Litter 1	63	2	—	—	—	1
2	95	6	6	2	2	2
3	131	1*	—	—	—	—
4	159	1*	—	—	—	—
5	182	—†	—	—	—	—
Killed	182					

All the offspring were externally female. The *Tfm*/Y offspring were classified as such by the finding of testes on dissection. There is no reason to link the second animal's poor rearing ability with *Tfm/0* condition, since normal mice often fail to rear litters of only one or two young, through insufficient stimulus to lactation.

* Pregnancy diagnosed by palpation; one foetus, but no live young seen. Foetus presumed stillborn.

† Pregnant when dissected; three dead implants only.

The ovaries of the older *Tfm/0*, killed at 9 months of age, were more abnormal (Fig. 2). The number of oocytes at all stages was markedly reduced, whereas that of corpora lutea was much increased, so that the bulk of the ovary consisted mainly of corpora lutea. But the normal ovarian architecture still remained and there was no sign of tumour or cyst formation. Such oocytes as there were appeared to be developing normally, up to stage 8 or 9 (ref. 7). The general picture was very similar to that found by Lyon and Hawker⁵ in a 9-month-old +/0 animal, and apparently was less severe than that found by Ohno *et al.*, in a *Tfm/0* of only 112 d. Thus, from our animals there is no evidence that a response to androgen is required for normal ovarian function.

The reasons for the discrepancy between Ohno's results and ours are not clear. Possible explanations include the following.

(1) Our animals were both with a male continuously from about 6 weeks of age. Therefore the ovaries will have been under the hormonal influence of corpora lutea of pregnancy, pseudopregnancy or lactation for most of the time. Ohno *et al.* suggested that perhaps this would tend to maintain ovarian normality. Pregnancy does not, however, affect the oocyte count in normal animals, although it does tend to prevent abnormalities of ovarian structure⁸. In order to explain the effects of *Tfm/0* mice on this basis therefore one might need to postulate that pregnancy protected the ovaries more strongly in them than in normal mice.

(2) Ohno *et al.*⁹ have found that the expression of the *Tfm* gene in the male can be altered by genetic factors on the X chromosome apparently similar to the controlling elements found by Cattana¹⁰. Possibly these elements also affect expression in females, and Ohno's stock and ours have come to differ in some unknown way in respect of them.

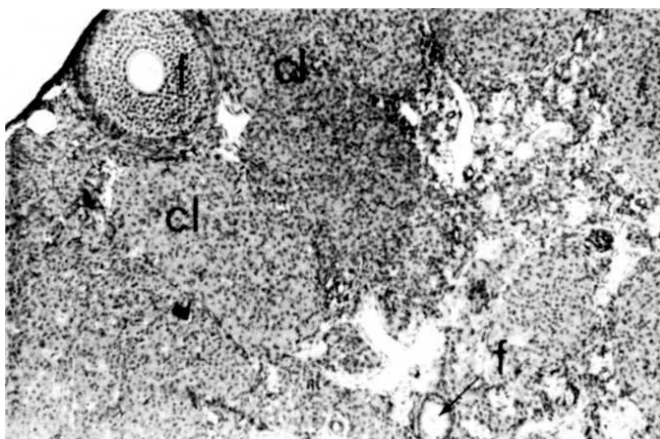


Fig. 2 Ovary of *Tfm/0* mouse, aged 9 months. Very few follicles (f), but numerous corpora lutea (cl). Both fixed in Heidenhain's Susa, stained haematoxylin and eosin. $\times 72$.

(3) Perhaps the general genetic background of the stock affected the expression of *Tfm/0* and +/0 in the ovaries. The numbers of oocytes, the age when all oocytes are depleted, and also changes with age in reproduction and ovarian structure vary markedly with strain in normal animals⁸ and so it would not be surprising if they also varied in XO mice.

It could, however, be that none of these explanations is correct, and until more is known there must remain some doubt concerning the importance of androgen in reproduction of female mice. We have shown that androgen is inessential for ovulation, mating, pregnancy and lactation. The results of Ohno *et al.* suggest that it is essential to maintain normal ovarian function; our own raise uncertainties about this point.

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Role of Hydrogen Bonding in Organic Cation Discrimination by Gallbladder Epithelium

THE main route of ion permeation across some epithelia, including that of the gallbladder, is by the so-called 'tight' junctions between adjacent cells¹. In the case of the gallbladder, this pathway is much more permeable to cations than to anions, and it also discriminates markedly among different inorganic cations^{2,3}. We have extended selective permeability measurements to a series of small amines in the form with one positive charge (compare ref. 4). The rationale behind these experiments is that permeability differences among cationic amines may help to identify the chemical nature and arrangement of ion-selective sites, as well as the possible existence of steric restraints in the permeation channel. The results indicate that gallbladder 'tight' junctions contain proton-acceptor sites which distinguish more markedly among cationic proton donors than does water.

Cation permeability ratios (P_x/P_{Na}) were measured as described previously^{2,3,5}, in flat sheets of bullfrog gallbladder mounted between two well-stirred chambers at 23°C. Briefly, transepithelial electrical potential differences (dilution potentials and biionic potentials) were measured with AgCl electrodes, and P_x/P_{Na} values were calculated by inserting these potentials into a Goldman-Hodgkin-Katz equation modified for the presence of a shunt³. For all amines studied, P_x/P_{Na} and gallbladder conductance were essentially constant throughout an experiment. Eighteen of the nineteen amines tested had pK_a values ≥ 9 and were studied at pH 7, while hydrazine (pK_a 8.1) was studied at pH 5. Thus, more than 99% of the dissolved amine was in the charged form. For amines with $pK_a \geq 9$, P_x/P_{Na} values measured at pH 5 and at pH 7 were found to be the same within experimental error.

Figure 1 displays the experimental values of P_x/P_{Na} , plotted as a function of the free-solution mobility ratios u_x/u_{Na} . For the nineteen amines as a group, it is obvious that P_x/P_{Na} neither equals, nor is a monotonic function of, u_x/u_{Na} . P_x/P_{Na} values are not even approximately in the sequence of reciprocal ionic sizes or of pK_a values. Figure 1, however, does exhibit two simple patterns. First, if one compares solutes with similar u_x/u_{Na} values, P_x/P_{Na} increases with the number of protons (≤ 1 , 2, 3, or ≥ 4) that the solute has available for hydrogen bonding with a suitable proton acceptor. For example, among amines with u_x/u_{Na} in the range of 0.65 to 0.80, the P_x/P_{Na} sequence is 0.55 for $H_2N^+=C(NH_2)CH_3$ (four donor protons); 0.35 for $H_3N^+-CH_2-CH=CH_2$ and 0.33 for $H_3N^+-CH_2-CH_2-CH_3$ (both with three protons); 0.18 for $H_3C-NH_2^+-CH_2-CH_3$ (two protons); and 0.02 or less for $(H_3C)_4N^+$ (no proton). The second pattern is that, among amines with a given number of donor protons (≤ 1 , 2, 3, or ≥ 4), P_x/P_{Na} values fall in the same or approximately the same sequence as u_x/u_{Na} values. For solutes with ≤ 1 proton, P_x/P_{Na} is too low to measure (that is, ≤ 0.02), regardless of the u_x/u_{Na} value.

Thus, there are four 'levels' of permeability, depending on the solute's available number of donor protons. Within each 'level', differences in molecular size or shape generally affect gallbladder P_x/P_{Na} values and free-solution u_x/u_{Na} values in the same sequence.

This relation between permeability and hydrogen-bonding ability indicates that gallbladder 'tight' junctions contain proton-acceptor sites, which distinguish more markedly among cationic proton donors than does water. Let us represent a solute's hydration energy as ΔF_{hyd} ; its attraction energy to membrane sites (mainly due to hydrogen-bond forces, which include electrostatic forces), as ΔF_{mem} ; the free-energy change in transferring solute from water to membrane sites, as ΔF_{trans} ; and the solute's partition coefficient between membrane sites and water, as k . Then $\Delta F_{trans} = \Delta F_{mem} - \Delta F_{hyd} = -RT \ln(k)$. If, for two solutes a and b, b having more

donor protons than a, the difference between the bonding energies in the membrane is greater than the difference in water (that is, $\Delta F_{mem,b} - \Delta F_{mem,a}$ more negative than $\Delta F_{hyd,b} - \Delta F_{hyd,a}$), then the membrane/water partition coefficient k will be larger for the solute b with more donor protons. Assuming (in analogy with other systems^{6,7}) that increased solute/membrane forces will increase the solute's partition coefficient by a factor larger than the factor by which they reduce the solute's mobility in the membrane, the net effect would be that the solute with more donor protons will have the larger permeability. The absence of higher permeability 'levels' than the level associated with four donor protons suggests that ΔF_{trans} is not increased by addition of further donor protons beyond four. Comparison of enthalpies of solution for alkylammonium ions with varying numbers of donor protons, in the solvents water, dimethyl sulphoxide, and propylene carbonate⁸, indicates that a dimethyl sulphoxide or propylene carbonate 'membrane' would resemble the gallbladder in its selective permeability to these cations. Note that membrane ligands need not necessarily form stronger H-bonds than water (ΔF_{mem} more negative than ΔF_{hyd}) to yield the pattern of Fig. 1; the ligands need only discriminate more among H-bond donors than does water ($\Delta F_{mem,b} - \Delta F_{mem,a}$ more negative than $\Delta F_{hyd,b} - \Delta F_{hyd,a}$).

The sodium channel of myelinated nerve⁴, and lipid bilayers doped with any one of four carriers⁹ (nonactin, trinactin, valinomycin, perhydroantamanide), resemble gallbladder in that selective permeability patterns exist for cationic amines

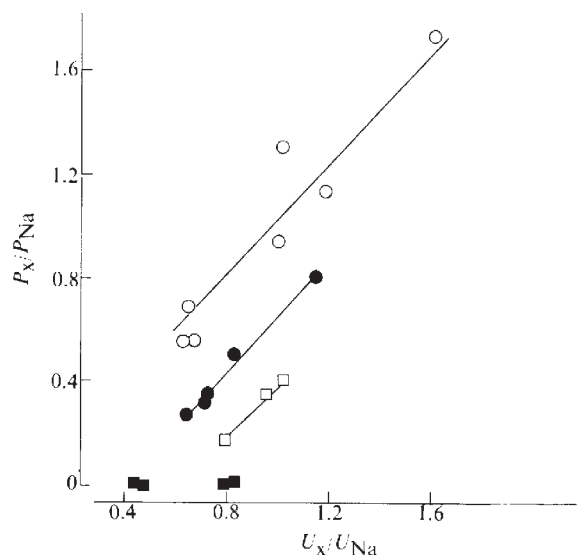


Fig. 1 Relative permeability coefficients in bullfrog gallbladder (P_x/P_{Na} , ordinate), as a function of relative free-solution mobilities (u_x/u_{Na}), for nineteen cationic amines in the form with one positive charge. P or u for a given cation (x^+) is expressed relative to P or u for Na^+ . Each point represents the average value of P_x/P_{Na} for three to eight gallbladders. The number of donor protons that each cation has available for hydrogen-bond formation is, $\circ$, ≥ 4 (NH_4^+ , $H_2N^+=CH-NH_2$, $H_2N-NH_3^+$, $(H_2N)_2C-NH_2^+$, $H_3C-NH(NH_2)C-NH_2^+$, $H_2N-NH^+=C(NH-NH_2)_2$, $H_3C(NH_2)C-NH_2^+$); $\bullet$, 3 ($H_3C-NH_3^+$, $H_3C-CH_2-NH_3^+$, $H_2C=CH-CH_2-NH_3^+$, $H_3C-CH_2-CH_2-NH_3^+$, $(H_3C)_2CH-NH_3^+$); $\square$, 2 ($H_2C \begin{smallmatrix} CH_2 \\ | \\ NH_2^+ \end{smallmatrix}$, $(H_3C)_2NH_2^+$, $H_3C-NH_2^+-CH_2-CH_3$); $\blacksquare$, 1 or zero, $(H_3C)_3NH^+$, $(H_3C)_4N^+$, $(C_2H_5)_3NH^+$, $(C_2H_5)_4N^+$. The straight lines are regression lines fitted through each set of points, without attributing theoretical significance to the fit. u_x/u_{Na} values were calculated from measured conductances of 150 mM amine chloride (x^+Cl^-) solutions, assuming Λ_{Cl^-} to be the same as in a 150 mM KCl solution.

and are related to their H-bonding ability. Of the amines tested in these five systems as well as in gallbladder, the most permeant species are the proton-rich NH_4^+ , $\text{H}_2\text{N}-\text{NH}_3^+$, $\text{H}_2\text{N}-\text{HC}=\text{NH}_2^+$, and $(\text{H}_2\text{N})_2\text{C}=\text{NH}_2^+$ in all six cases. (The permeability sequence of these proton-rich amines differs, however, among the six systems, due probably to small differences in factors such as the strength of site/solute interactions. The close agreement in sequence, within a given proton 'level', between P_x/P_N for gallbladder and u_x/u_{Na} may be coincidental.) For the carriers nonactin and valinomycin, as for gallbladder, it was shown⁹ by comparison of alkyl-substituted ammonium derivatives that removal of each successive proton of NH_4^+ reduces permeability. The main difference between the results for gallbladder and the other five systems is quantitative: the range of selectivity is much narrower in gallbladder. Thus, the relative permeability coefficients for NH_4^+ , $\text{H}_3\text{C}-\text{NH}_3^+$, $(\text{H}_3\text{C})_2\text{NH}_2^+$, and $(\text{H}_3\text{C})_3\text{NH}^+$, respectively, are 1.00, 0.47, 0.20, ≤ 0.01 in gallbladder; 1.00, 0.0088, 0.00075, 0.000125 in valinomycin-doped bilayers; and 1.00, 0.00013, 0.00001, 0.000004 in nonactin-doped bilayers. By analogy with similar results in model systems¹⁰, this finding indicates that the tight junctions of gallbladder provide a more hydrated environment than do carriers or the Na^+ channel of nerve, as was also concluded from analysis of alkali cation selectivity¹⁻³.

Figure 1 also illustrates how easy it is to be misled about the permeability pattern if only a restricted group of solutes is studied. For example, if only amines with the same number of donor protons are compared, the parallel between permeability ratios and free-solution mobility ratios would suggest that gallbladder is completely non-selective. Similarly, comparison only of alkylammonium ions such as NH_4^+ , $\text{H}_3\text{C}-\text{NH}_3^+$, $(\text{H}_3\text{C})_2\text{NH}_2^+$, and $(\text{H}_3\text{C})_4\text{N}^+$ might suggest that selectivity results from molecular sieving in a narrow channel. In fact, an effective pore radius cannot be estimated simply from the size of the smallest impermeant amine, as permeability depends upon H-bonds as well as on size. Thus, $(\text{H}_3\text{C})_4\text{N}^+$ is virtually impermeant, while $\text{H}_2\text{N}-\text{NH}^+=\text{C}(\text{NH}-\text{NH}_2)_2$, which is twice as large (but has many more donor protons), is quite permeant ($P_x/P_{\text{Na}}=0.55$). Again, $(\text{H}_2\text{N})_2\text{C}=\text{NH}_2^+$, $(\text{H}_3\text{C})_2\text{CH}-\text{NH}_3^+$, and $(\text{H}_3\text{C})_2\text{NH}^+-\text{CH}_3$ have approximately the same size and shape, but the respective P_x/P_{Na} values are 0.94, 0.27, and ≤ 0.02 , in the sequence of number of protons (six, three and one, respectively). To be able to detect any steric restriction, one must first recognise the pattern arising from H-bonds in the absence of steric effects. Differences between the bullfrog gallbladder results reported here and our unpublished permeability determinations for the same cationic amines in rabbit gallbladder seem to be due mainly to more marked steric restriction in the rabbit.

Finally, it is interesting to note that gallbladder permeability coefficients of amines in the uncharged form, and of most other non-electrolytes, decrease with increasing H-bonding ability^{7,11}. Lipid-soluble non-electrolytes presumably cross gallbladder epithelium through the cell membranes rather than through the tight junctions, and a similar inverse relation between permeability and H-bonding ability is observed for membranes of single cells and for lipid bilayers. Such a pattern is exactly the opposite of the pattern described here for amines in the charged form permeating by tight junctions, where permeability coefficients increase with increasing H-bonding ability. Evidently the selectivity of tight junctions arises from the properties of their H-bonding sites, whereas the selectivity of cell membranes arises from the paucity of H-bonding sites in the bilayer interior compared with water^{7,11}. Permeability studies with organic cations may prove similarly useful for characterising the molecular environment in other pore-like permeation pathways in biological systems.

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Intra- and Interpopulation Selection concerning the Alcohol Dehydrogenase Locus in *Drosophila melanogaster*

Lewontin and Hubby¹ have shown, by means of protein electrophoresis, that about one third of the loci in *Drosophila pseudoobscura* populations are polymorphic. Comparable studies by others have provided similar figures for a variety of species including man^{2,3}. Some authors⁴⁻⁶ have argued that the observed protein variation is merely a product of mutation and drift of neutral variants and is not maintained by selection. This view is severely criticised by others^{7,8}, who stress the importance of selective forces. Indications for the operation of selective forces have mainly come, until now, from studies on geographical variation^{9,10}. Direct experimental evidence, concerning the relative selective values of electrophoretic variants is, however, scarce. In one well-documented case, Yamazaki¹¹ did not find selective differences between esterase-5 variants in *D. pseudoobscura*.

To obtain more relevant information on the nature of protein variation we studied the widespread polymorphism of the alcohol dehydrogenase locus in *D. melanogaster*. The *Adh*-locus is located on the second chromosome (2-50.1) and two alleles (*Adh*^s and *Adh*^F) are known from natural populations¹². To characterise *Adh*-genotypes agar gel electrophoresis was carried out on whole fly homogenates for 1 h at 140 V and 28 mA in 0.02 M veronal buffer, pH 8.5. After electrophoresis the gels were transferred to a solution consisting of 97 ml 0.1 M veronal buffer pH 8.5, 3 ml ethanol 96% and 50 mg NAD. After 15 min the sites of alcohol dehydrogenase activity on the gel can be distinguished by fluorescence under ultraviolet light (366 nm). We applied the nomenclature of Grell¹³ for the description of the slow and fast alleles. Five homozygous S-lines and five homozygous F-lines were isolated from the polymorphic Groningen population by making single pair matings. Each homozygous line originated from a mating in which both parents were homozygous for the same allele. The Groningen base population was founded three years before with several inseminated females collected at a local fruit market and since kept in the laboratory as a cage population with several thousands of individuals. The frequency of the F-allele fluctuated between 0.50-0.65 during that time.

To investigate whether selection occurs, cage populations

Table 1 Change in frequencies of the *Adh^F*-allele in cage populations (based on samples of seventy-five ♀♀ and seventy-five ♂♂)

Initial frequency	Frequency after 5 months	Frequency after 19 months
0.20	0.37	0.66
0.20	0.55	0.54
0.40	0.55	0.59
0.40	0.54	0.63
0.60	0.55	0.45
0.60	0.65	0.64
0.80	0.60	0.60
0.80	0.64	0.68
0.62*	0.53	0.54

* Groningen base population.

were started with different allelic frequencies (0.20, 0.40, 0.60 and 0.80 F). The rationale of this experiment is that in the case of neutral alleles, the frequencies will show small fluctuations around the initial values, and should differential selection work, there will be determinate changes. Each population was started with 200 pairs of flies from the homozygous S- and F-lines in the appropriate proportions; each initial frequency was represented by two cages. Allelic frequencies were determined twice: 5 and 19 months after the start of the experiment. For this purpose, fresh food cups were inserted in the cages for one day, and from the flies emerging from these cups, a sample of seventy-five ♀♀ and seventy-five ♂♂ was electrophoresed for genotype identification. Marked changes in allelic frequencies occurred after 5 months (approximately ten generations) (Table 1). An increase is observed in the 0.20 and 0.40 F populations, a decrease is found in the 0.80 F population, as in the 0.60 F populations no distinct changes occur. A second sample of flies, taken from the cages after 19 months (approximately thirty-eight generations), shows allelic frequencies lying between 0.45–0.68 F.

Another experiment was designed to measure the survival of populations, differing in genetic composition with regard to the *Adh*-locus, in more or less extreme environments. For this purpose four kinds of populations were started in vials using flies from the homozygous S- and F-lines: monomorphic F; monomorphic S; polymorphic with a frequency of 0.20 F; and polymorphic with a frequency of 0.80 F. Each of these four population types was represented with 50 vial populations in each of the nine environments listed in Table 2. Each vial population was started with ten females, mated previously with the appropriate males. The following procedure was then applied: the parents were removed and discarded before the emergence of their offspring, all flies from the new generation were shaken into vials with fresh food, without mixing between vials. This was repeated each generation, the time of transfer varying between environments because of the differences in development time. Extinction is defined as the proportion of vials which fail to produce at least one pair of offspring in a given generation.

The outcome of the extinction experiments is given in Table 2, where cumulative extinction percentages after eight generations are listed. It seems that the severity of the environmental stress differs considerably among environments. Table 2 also shows that, though in some environments there are no differences in survival, population types differ greatly in extinction frequency in others. A test of independence shows that extinction frequency is not independent of population type in environments 5, 6, 7, 8 and 9. The monomorphic S population type shows, relative to the other population types, the highest extinction rate on ethanol-supplemented medium and at low temperature. This population type shows on the contrary, the lowest extinction rate in high humidity conditions, where the monomorphic F type has the highest extinction rate. At high temperature, both

polymorphic population types have a lower extinction rate compared with the monomorphic ones, the opposite is true in low humidity conditions.

In addition to measuring extinction, allelic frequencies were determined in both types of polymorphic population types in environments 1, 2, 4, 5 and 6. For this purpose, a variable number of flies derived from ten randomly chosen vials were electrophoresed (Table 3). In all but one of the investigated environments identical allelic frequency changes occur: the 0.20 F populations show an increase to values >0.40 F, the 0.80 F populations a decrease to values between 0.60 and 0.70 F. The one exception is the ethanol-supplemented medium, in which the 0.20 F populations show a much more pronounced rise and the 0.80 F populations also show an increase.

What do these findings mean for the selection *versus* drift controversy? The determinate changes in allelic frequencies in the cage populations, leading to final frequencies very close to the Groningen base population, strongly point to some kind of balancing selection. Neutrality of the alleles would only lead to small chance fluctuations around the initial values, as population sizes amount to several thousands. When the observed numbers of genotypes, from which the allelic frequencies in Table 1 are calculated, are compared with the numbers, expected on the basis of the Hardy-Weinberg equilibrium, significant deviations ($P < 0.05$) are found in seven of eighteen cases. In all these cases, the

Table 2 Cumulative extinction percentages in different environmental conditions after eight generations

Population type	Environments								
	1	2	3*	4	5	6	7†	8	9
Monomorphic F	4	90	100	36	28	94	8	8	28
Monomorphic S	0	94	100	24	62	92	32	14	2
Polymorphic (0.20 F)	10	82	100	36	30	54	12	30	10
Polymorphic (0.80 F)	6	78	100	42	20	66	12	20	18
χ^2 ‡	5.5	6.7		3.8	22.6§	32.4§	13.1§	8.9¶	15.0§

* Complete extinction after four generations.

† Extinction percentages after three generations.

‡ χ^2 for independence.§ $P < 0.01$.¶ $P < 0.05$.

The following environmental conditions were applied. 1, Control, temperature 25° C, 70% R.H., food medium consisting of 1,000 ml water, 19 g agar, 54 g sucrose, 32 g yeast, 13 ml nipagin solution (10 g nipagin in 100 ml ethanol 96%); 2, yeast amount reduced to 5 g; 3, yeast amount reduced to 5 g, medium supplemented with 10% ethanol; 4, sucrose content reduced to 25 g, after 6 generations further reduced to 12.5 g; 5, medium supplemented with 20% ethanol; 6, temperature 29.8° C; 7, temperature 15° C; 8, 35% R.H.; 9, 95–100% R.H.

For conditions 2–9 only differences relative to the control are listed.

actual number of heterozygotes exceeds the expected number. Detailed fitness measurements, now in progress, will elucidate the exact nature of the selection process. It cannot be stated with absolute certainty, however, from these experiments that the *Adh*-locus itself is involved, instead of a closely linked locus. In that case, an initial linkage disequilibrium between the *Adh*-locus and the linked locus may bring about the described reaction. The rapid rise in F-frequency, however, found in the vial populations with medium supplemented with ethanol, which afterwards proved to be a general phenomenon for polymorphic *D. melanogaster* populations from different geographic origins (Van Delden, unpublished), strongly suggests a reaction of the *Adh*-locus itself on a relevant environmental change. Recently¹³, comparable systematic changes in allelic frequencies, follow-

ing changes in the composition of the food, were found for the amylase locus in *D. melanogaster*.

The extinction experiments show that the genetic composition of a population concerning the *Adh*-locus, at least in some environments, strongly influences the chance of

morphism is the overall best adaptive strategy in varying environments, the development of systems providing balanced polymorphism will be stimulated.

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Table 3 Change in frequencies of the *Adh^F*-allele in polymorphic vial populations, kept in different environments

Environ- ments*	Genera- tion	0.20 F populations	0.80 F populations
1	7	0.42 (250)	0.65 (234)
2	4	0.43 (168)	0.70 (192)
3	3	0.42 (84)	0.62 (62)
5	5	0.65 (224)	0.87 (243)
6	5	0.42 (282)	0.67 (300)

* As in Table 2.

Sample size given in parentheses.

population survival. Different environments favour different population types. Experimental data on the relative success of populations, differing in genetic composition, are scarce. Studies on third chromosome inversion polymorphism in *D. pseudoobscura* have shown that polymorphic populations are superior to monomorphic ones, in such characteristics as population size and innate capacity of increase^{14,15}. Though in the present experiment the polymorphic population types show the lowest extinction in the high temperature environment, this is, as the data show, no general phenomenon holding for all environments. It is interesting to note that for some environmental conditions there is a relation between the extinction rate of population types and the specific activities of the *Adh*-genotypes concerned. Gibson¹⁶ found that after heat treatment of larval extracts, the heterozygotes possess the highest remaining activity, as in our experiments, polymorphic populations show the lowest extinction rates. Further, he found that when larvae are cultured on food, supplemented with ethanol, the SS genotypes, which have the lowest activity in normal conditions, show less increase in activity than the other genotypes. This corresponds with our findings that the SS monomorphic populations show the highest extinction rates on this medium.

Although the results of our experiments strongly point to the occurrence of some kind of balancing selection, we further envisage a situation in which selection between populations is working, superimposed on the selection process within populations. Lewontin¹⁷ has discussed the relations between such intra- and interpopulation selection, and stressed the importance of interpopulation selection for the evolution of genetic systems. The fossil record shows that extinction of species and higher systematic categories is a widespread phenomenon and inextricably linked with the evolutionary process, but we do not know if extinction of local populations, and more specifically of *Drosophila* populations, plays an important role in nature. It is quite probable, however, that extreme temperature and humidity conditions, shortage of certain food components, and excess of harmful products, for example, create a severe stress on local populations, leading to complete extinction of part of them. If the probability of extinction is correlated with genotype composition, the random succession of a number of differing extreme environments would provide polymorphic populations, segregating for all genotypes, with the best chances of long-term survival. A survey of natural populations would then show most of them to be polymorphic. Relevant to this conclusion is the finding that for experimental *D. willistoni* populations genetic variability is higher in populations kept in heterogeneous environments than in populations in more constant environments¹⁸. It is tempting, though speculative, to assume that when poly-

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Synergistic Response between Thymus and Lymph Node Cells in the Mixed Lymphocyte Culture

In rodents the mixed lymphocyte culture (MLC) reaction probably represents an *in vitro* model of the graft versus host (GvH) response¹. Thymus-dependent lymphoid cells (T cells) are thought to be responsible for both reactions^{2,3}. Cantor and Asofsky^{4,5} presented evidence for the synergistic interaction of two thymus-dependent lymphocyte populations in the GvH response. In their studies one cell type (amplifier cells or T₂ cells) was obtained from the peripheral circulation, the other type (precursor cell or T₁ cell) was found in greatest concentration in the thymus and spleen. A mixture of thymocytes and peripheral blood lymphocytes produced a GvH reaction significantly greater than was expected from summation of the separate reactivities of the components of the mixture. This 'synergy' occurred only when both cell populations had been obtained from donors allogeneic to the host⁴.

We present here evidence that the *in vitro* 'one way' MLC reaction can also be used to show 'synergy' between subpopulations of lymphocytes. For our experiments, 4 to 8-month-old C57Bl/6J (B6) and CBA/6J (CBA) and 2-month-old (B6×CBA) F₁-hybrid males were used. Thymuses, lymph nodes (LN), and spleens were removed aseptically. Cell suspensions free of debris were prepared in HEPES-buffered medium RPMI/1640 (Gibco) containing 1% antibiotic-antimycotic-mixture (Gibco) and 7% pooled, heat-inactivated normal human serum. Spleen cell suspensions were treated with Tris-buffered ammonium chloride to remove contaminating red blood cells⁶. For the MLC reaction, 0.1 ml of the responding cell suspension was incubated for 48 h at 37° C in a humidified atmosphere with 0.1 ml of mitomycin C-treated (mito-treated)⁷ stimulating

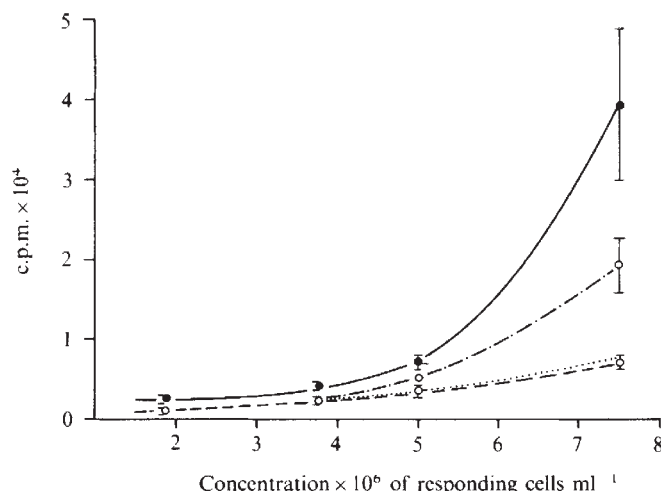


Fig. 1 ^3H -thymidine uptake (in c.p.m.) of LN cells (—), thymocytes (---), and a mixture of 20% LN cells 80% thymocytes (— · — · —), from 6-month-old B6 mice, in relation to the concentration of responding cells. The ratio of stimulating cells (mito-treated CBA spleen cells) to responding cells was 1 to 1 in each case. The 'expected' reactivity for the LN-thymocyte mixture (···) was calculated from the reactivity curves for equivalent absolute numbers of LN cells and thymocytes. This calculated sum of c.p.m. values of the participating partner cells was significantly less than the observed ^3H -thymidine uptake of the mixture. The vertical bars in this and the other figures represent standard error.

cell suspension in the U-shaped cups of titration plates (Scientific Products; 220-24A Disco U titration plates) which had been sealed with covers (Linbro, 35 PSM). Then $3 \mu\text{Ci}$ of ^3H -thymidine (Schwarz Mann; specific activity 1.0 Ci mmol^{-1}) in 0.1 ml of serum-free medium were added to each cup. After a further 15 to 18 h of incubation, the reaction was stopped by chilling, the reaction mixture transferred to centrifuge tubes, washed twice in 5% trichloroacetic acid, once in 100% methanol, transferred quantita-

tively to counting vials, and evaporated to dryness. Scintillation liquid (5 g PPO and 100 mg POPPOP 1^{-1}) containing 3% solubiliser (Nuclear-Chicago Corp.) was added to each tube and the activity measured in a Beckman model LS-250 liquid scintillation counter. The amounts of ^3H -thymidine uptake in tubes containing only synergistic partner cell were used as controls and subtracted from the experimental (allogeneic) values. Final values were expressed as c.p.m. $\times 10^4$. Each value in Figs 1, 2 and 3 and Table 1 represents the arithmetic mean with standard error from four separate cultures. Student's t test was used for statistical comparisons. When 'expected' compared with mean values were compared the s.e. of the observed value was used for both means: this introduced a slight conservative bias in the probability values.

Figure 1 shows results for different concentrations of responding cells of separate MLC-reactions for LN cells, thymocytes, and a mixture containing 20% LN cells and 80% thymocytes from B6 mice. Mito-treated CBA spleen cells were used as stimulating cells. LN cells showed much greater ^3H -thymidine uptake than thymocytes. The LN-thymocyte mixtures reacted more strongly than expected: according to the curves, 1.5×10^6 LN cells ml^{-1} ($=20\%$ of 7.5×10^6) should give about 0.25×10^4 c.p.m. and 6×10^6 thymocytes ml^{-1} ($=80\%$ of 7.5×10^6) about 0.4×10^4 c.p.m. The sum of both c.p.m. values would therefore equal 0.65×10^4 c.p.m. Actually, 7.5×10^6 cells of the 20% / 80% mixture showed 1.95×10^4 c.p.m., which is about three times the 'expected' value ($P=0.01$).

In the experiment shown in Fig. 1 equal total numbers of stimulating and responding cells were present in each cup. It might therefore be argued that the LN cell component of the LN-thymocyte mixture received extra stimulation from the increased numbers of stimulating CBA cells per LN cells present in the mixture. The apparent 'synergy' might in this case be spurious. Figure 2 shows results of MLC-reactions in which the concentration of stimulating cells in each cup was kept constant, and so the ratio of responding cells to stimulating cells increased with increasing concentration of responding cells. Here the reactivity of the LN-thymocyte mixture at the responding cell concentration of $7.5 \times 10^6 \text{ ml}^{-1}$ was about four times the 'expected' value ($P=0.05$).

Figure 3 includes results of an experiment in which the ratio of LN to thymus cells in the responding cell mixture was allowed to vary but the total concentration of the responding cells was kept at 7.5×10^6 cells ml^{-1} for each cup. It also shows the results when one of the cells in the LN-thymocyte mixture was inhibited by previous treatment with mitomycin. Separate experiments demonstrated that mito-treated syngeneic partner cells behaved like 'innocent bystanders' and did not affect the reactivity of the untreated partner cells. The 'expected' curve in Fig. 3 was constructed from the reactivity curves of the mixtures LN_{mito}-thymocyte and LN-thymocyte_{mito}. The untreated LN-thymocyte mixture demonstrated a statistically significant synergistic effect when LN cells constituted 20 to 40% of the mixture. Mitomycin-inhibited cells were not able to synergise.

Table 1 gives results of experiments comparing MLC reactions of LN and thymus cells of B6 mice as responding cells with reactions where one or both responding cell came from (B6 $\times$ CBA) F_1 hybrids. Stimulating cells were CBA in all cases. Now F_1 hybrid cells are not normally stimulated in the MLC reaction. These experiments were therefore set up to determine if F_1 hybrid cells could participate in synergy. The highest ^3H -thymidine uptake ($42,274$ c.p.m.; control not subtracted) for any of the mixtures was seen when both lymph node cells and thymocytes were derived from B6 mice (Table 1). The LN-thymocyte mixture in which both cells was derived from the hybrids showed the lowest c.p.m. value ($4,494$ c.p.m.). This value was, however, higher than for a syngeneic control in which the same LN-thymocyte mixture was allowed to react against mito-treated

Table 1 MLC reactivity of LN and thymus cells and of parental and parental-hybrid mixture

Source of resp. cells.	Strain	Cell concentration ($\times 10^6$)	c.p.m.	
			Allogeneic	Syngeneic
LN	B6	7.5	$114,949 \pm 5,400$	1,232
LN	B6	3.75	$67,740 \pm 8,200$	484
LN	B6	1.875	$16,467 \pm 6,450$	—
LN	B6	0.9375	$4,411 \pm 1,900$	—
Thymus	B6	7.5	$11,619 \pm 620$	289
Thymus	B6	3.75	$1,425 \pm 200$	248
				—
LN 20% Thymus 80%	B6 } B6 }	7.5	$19,000^*$	—
LN 20% Thymus 80%	B6 } B6 }	7.5	$42,274 \pm 4,800$	1,308
LN 20% Thymus 80%	B6 $\times$ CBA } B6 $\times$ CBA }	7.5	$4,494 \pm 700$	727
LN 20% Thymus 80%	B6 } B6 $\times$ CBA }	7.5	$31,569 \pm 2,800$	2,223
LN 20% Thymus 80%	B6 $\times$ CBA } B6 }	7.5	$13,256 \pm 4,600$	2,179

* B6 LN/B6 thymus, expected compared with observed, $P < 0.01$; B6 LN/B6 thymus expected compared with B6 LN/(B6 $\times$ CBA) thymus observed, $P < 0.02$; B6 LN/B6 thymus observed compared with B6 LN/(B6 $\times$ CBA) thymus observed, $P = 0.01$.

† Expected value for the LN-thymus mixture.

MLC reactivities of B6-LN cells and B6 thymocytes at different cell concentrations, and of 20/80 LN-thymocyte mixtures at a total concentration of 7.5×10^6 cells per ml. The cells in the mixtures were derived from B6 mice or (B6 $\times$ CBA) hybrids. Mito-treated CBA spleen cells (7.5×10^6 cells per ml) served as stimulating cells. An optimal (synergistic) response was observed only when both partner cells could be stimulated, that is they were from B6 mice.

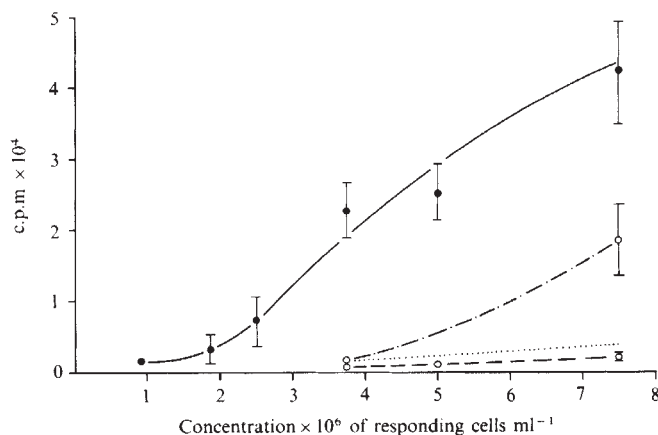


Fig. 2 Legend as for Fig. 1, except that the concentration of the stimulating mito-treated CBA spleen cells was kept constant at 7.5×10^6 cells ml^{-1} ; and so the ratio of responding to stimulating cells increased with increasing concentration of responding cells. The reactivity of the LN-thymocyte mixture at the responding cell concentration of 7.5×10^6 cells ml^{-1} was about four times the 'expected' value.

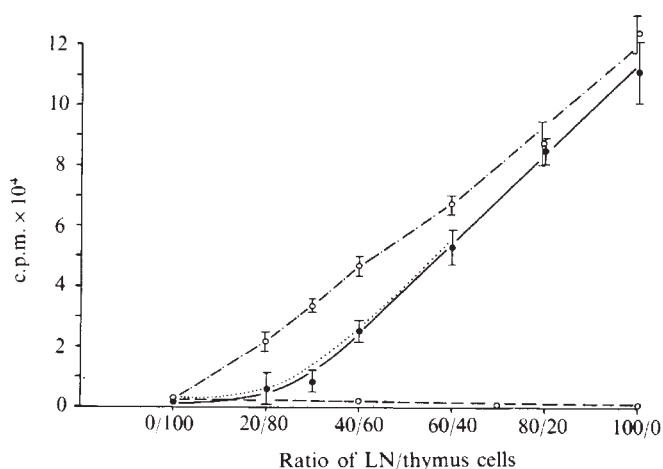


Fig. 3 MLC reactivities of LN cells and thymocytes (— · — · —), LN cells and thymocytes_{mito} (—) and thymocytes and LN cells_{mito} (---) at varying mixture ratios but with a constant total concentration of responding cells (8×10^6 cells ml^{-1}). The expected reactivities for the LN-thymus mixture (· · ·) were calculated from the reactivity curves for LN-thymocytes_{mito} and thymocytes-LN_{mito}. The observed reactivities of the LN-thymocyte mixtures at ratios of 20:80, 30:70 and 40:60, respectively, were significantly greater than these 'expected' reactivities. Synergy was not observed when one participating partner of a mixture had been treated with mitomycin. The concentration of stimulating mito-treated CBA spleen cells was 8×10^6 ml^{-1} .

syngeneic (B6 × CBA) F_1 hybrid cells (c.p.m. = 4.494 for allogeneic compared with 727 for syngeneic cells, Table 1). This suggests that a weak reaction occurred in the combination (B6 × CBA) LN-thymocyte responding cells compared with mito-treated CBA spleen cells. Boehmer and Adams⁸ found that mito-treated CBA spleen cells stimulated syngeneic CBA thymus cells weakly. It seems likely that the thymocyte component of the (B6 × CBA) LN-thymocyte mixture might have been stimulated by CBA spleen cells. (Boehmer and Adams⁸ did not find a syngeneic interaction of cells derived from B6 mice, but only from CBA mice.) The (B6 × CBA) LN and B6-thymocyte mixture showed reactivity numerically higher but not statistically significantly different from pure B6 thymocytes (Table 1). The B6-LN-(B6 × CBA)-thymocyte mixture exceeded the 'expected'

reactivity of the B6-LN-B6-thymocyte mixture. This might be explained by a reaction of hybrid thymocytes with mito-treated CBA spleen cells, as noted above. This reaction, however, was still significantly lower ($P < 0.02$) than the actually observed (synergistic) ^3H -thymidine uptake of the B6-LN-thymocyte mixture. It is additionally possible that in the parental-hybrid mixtures the parental cells received some stimulation by the extra antigens of the hybrid component⁹, which also could account for somewhat higher syngeneic control values (2,223 and 2,179 c.p.m., Table 1). These values were somewhat higher than the control values for pure LN cells or pure thymus suspensions. Thus, in the allogeneic system parental cells of a parental-hybrid mixture might be stimulated to a mild degree both by the mito-treated CBA spleen cells and by the cells of the hybrid partner. In general, however, our experiment indicates that an optimal (synergistic) response occurs only when both partners of the responding LN-thymocyte mixtures recognise the stimulating cells as foreign.

Our finding of a synergistic response involving LN and thymus cells in the MLC reaction seems analogous to the results of Cantor and Asofsky³⁻⁵ with the GvH reaction. A maximum response occurred in the LN-thymocyte mixture when the LN cells comprised 20 to 40% of the responding cell population. Mitomycin-inhibited partner cells were not able to participate in synergy. Synergy did not seem to occur to any great degree unless both cell populations were obtained from donors allogeneic to the stimulating cells. In experiments not detailed here treatment of LN cells with anti- θ serum abolished synergy. The MLC reaction is considered by most investigators to be the *in vitro* counterpart of the GvH reaction. Our results may be interpreted to support this view and suggest that for optimal responses the same cell subpopulations may interact both in the MLC reaction and the GvH reaction.

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Proliferative Capacity of Human Alveolar Macrophage

THE alveolar macrophage plays a crucial role in the pulmonary ecology and is a first line of cellular defence for the lung^{1,2}. Although animal studies indicate that pulmonary macrophages originate from the bone marrow³⁻⁵, little is known of the origin or fate of these cells in man. Cigarette smokers, however, are known to have substantially increased numbers of macrophages in the lung^{1,6} and the pulmonary macrophages in smokers demonstrate characteristic morphologic and functional attributes^{1,2,7,8}.

To determine whether human alveolar macrophages have proliferative capacity, we obtained cells by bronchopulmonary lavage from normal cigarette smokers and a non-smoker and performed *in vitro* tritiated thymidine labelling and culture studies. The data obtained indicate that some of these cells have proliferative capacity and that the lung macrophage population may therefore be locally self-sustaining.

Pulmonary cells were obtained from five normal volunteers (four cigarette smokers and one non-smoker) by the procedure of bronchopulmonary lavage as previously described⁹. Briefly, under local anaesthesia and fluoroscopic control, a catheter was placed into a lower lobe bronchus and lavage performed with 300 ml of normal saline. The cells were collected in Hank's balanced salt solution containing 20% foetal calf serum and 200 U of penicillin and 100 μ g streptomycin ml⁻¹. The cells were recovered by centrifugation at 150g for 7 min, washed twice, and suspended in McCoy's 5A medium with 20% foetal calf serum and antibiotics¹⁰.

Labelling studies were performed 1 to 3 h after pulmonary lavage by exposing the cells to ³H-thymidine (specific activity 2 Ci mmol⁻¹) at a concentration of 1 mCi ml⁻¹ of cell suspension for 1 h (ref. 11). In blocking experiments, cytosine arabinoside (final concentration, 10⁻⁵ M) or hydroxyurea (10⁻³ M) was added to the cell suspension 30 min before radiothymidine exposure¹². After incubation with ³H-thymidine, the cells were washed twice in complete tissue culture medium and deposited on glass slides with a cyto-centrifuge. Slides coated with Kodak NTB emulsion were exposed for 7 d, developed, and stained with Giemsa¹¹. Labelling indices were expressed as % macrophages labelled with a minimum count of 500 cells.

Pulmonary cells were cultured using the *in vitro* diffusion chamber technique previously described^{10,11}. Differential cell counts were performed on Giemsa stained specimens and macrophage identification was also based on electron microscopy, histochemical reaction for lipase (alpha naphthyl butyrate substrate)¹³ and tests of functional capacity including phagocytosis of yeast and bacteria and tests for the presence of cell surface receptors for immunoglobulin¹⁴. Lymphocytes were identified by characteristic morphology at the light and electron microscopic level and transformed lymphocytes by intense staining with methyl green pyronin.

Table 1 summarises the observed ³H-thymidine labelling indices. The percentage of macrophages incorporating label was found to vary between 0.35 and 1.25. The labelled cells generally had the morphology of immature macrophages³ but occasional mature macrophages also incorporated thymidine (Fig. 1). Some of the labelled cells were seen to contain smoker particles thereby substantiating that label was taken up by phagocytic cells which had been residing in the airways (Fig. 1). Tritiated thymidine uptake was blocked more than 90% by either cytosine arabinoside or hydroxyurea indicating that labelling resulted primarily from scheduled DNA synthesis rather than DNA repair¹². Occasional mitotic figures (Fig. 2) in macrophages were also observed.

Table 1 Pulmonary macrophage recovery and ³H-thymidine labelling

Subject	Viable cells recovered ($\times 10^6$)	Macrophages (%)	Total macrophages ($\times 10^6$)	Macrophage labelling index (%)
Smokers	1 45	99	45	0.75
	2 62	97	60	1.25
	3 84	98	82	0.35
	4 160	92	147	0.5
Non-smoker 5	11	72	7.9	0.35

Differential and total cell counts (Table 1) confirmed that pulmonary macrophages retrievable by bronchopulmonary lavage are markedly increased in cigarette smokers. These cells had the typical morphology of smoker macrophages as

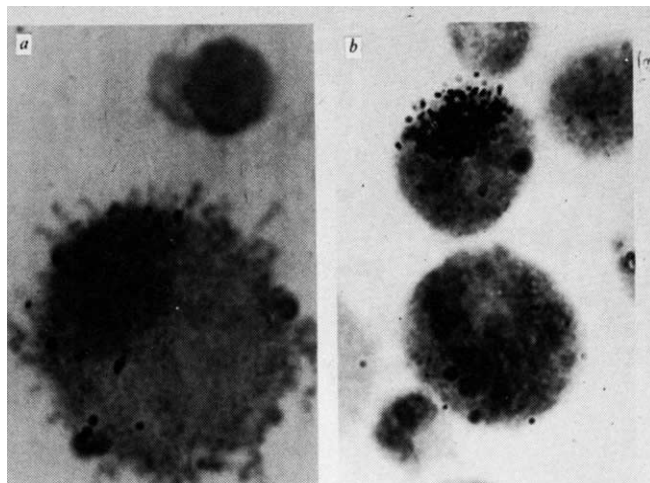


Fig. 1 *a*, Mature macrophage which has incorporated ³H-thymidine and a small lymphocyte from non-smoker; *b*, two macrophages with smoker inclusions. Upper cell has heavy nuclear label.

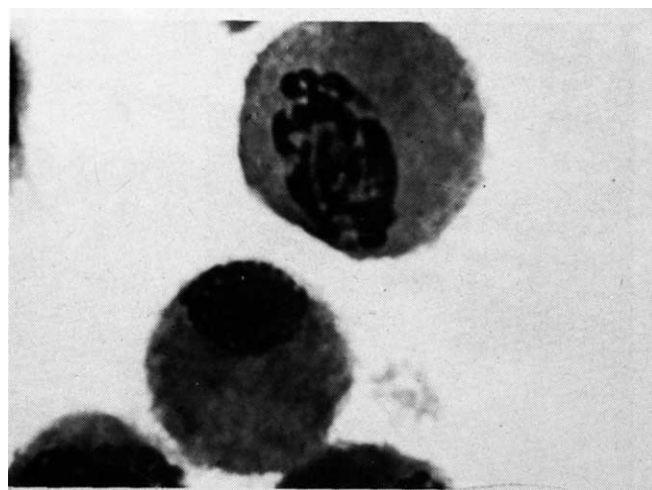


Fig. 2 Mitotic figure in pulmonary alveolar macrophage and non-mitotic cell of approximately the same maturity.

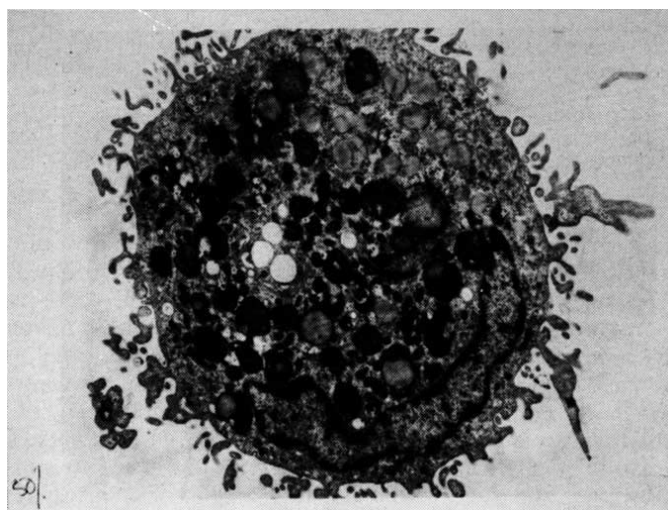


Fig. 3 Electron micrograph of pulmonary macrophage in culture at 14 d. Lipid inclusions and smoker particles are clearly seen.

viewed by light and electron microscopy^{7,8} and their morphological characteristics were maintained *in vitro* for several weeks (Fig. 3). They retained phagocytic capacity in culture for periods in excess of 50 d. Low labelling was also observed in macrophages maintained *in vitro* for up to 10 d and then exposed to ³H-thymidine for 1 h, indicating that these cells continued to synthesise DNA while in culture. In three experiments, the cells from smokers were exposed for 1 h to ³H-thymidine at the initiation of culture and then washed and grown in medium containing 10⁻⁵ M thymidine¹¹. In these cultures, labelled mature macrophages were observed for 5 to 14 d *in vitro*. Replicating transformed lymphocytes were also seen for several weeks in cultures from the non-smoker, indicating that lymphocytes resident in the lung are also capable of proliferation.

There is evidence from animal studies that pulmonary macrophages originate in the bone marrow³⁻⁵. Data are also available indicating that both the pulmonary alveolar macrophage and the hepatic macrophage can replicate *in situ*^{15,16}. It is presumed that in man the alveolar macrophages originate in the bone marrow and that the lung macrophage population is supported by an influx of monocytes from the blood. This concept raises important questions regarding the fate of the alveolar macrophage population in patients with bone marrow failure and severe leukopaenia. It also implies that needed increases in lung macrophages are dependent on a recruitment mechanism from the bone marrow¹⁰.

Our studies were undertaken to determine if the human pulmonary macrophage has proliferative capacity. The data obtained indicate that a small percentage of macrophages resident in the lung incorporate ³H-thymidine. This incorporation probably represents pre-replicative DNA synthesis for the following reasons: labelling was heavy and limited to a small percentage of cells; uptake of ³H-thymidine was blocked by hydroxyurea and cytosine arabinoside and mitotic figures were observed.

The long life of the pulmonary macrophage in culture lends support to the concept that these cells may be quite long-lived *in vivo*^{3,18}. A small growth fraction, therefore, as suggested by the low labelling index, could be sufficient to maintain a stable cell population without the need for an influx of mononuclear cells from the blood. This is consistent with data obtained for the pulmonary and hepatic macrophage in animals^{16,17} and general information regarding the proliferative capacity of mononuclear phagocytes¹⁸. Local pulmonary macrophage replication has also been shown to occur in rats exposed to NO₂ (ref. 15).

Our studies indicate that the human pulmonary macrophage is capable of replication. The macrophage compartment could, therefore, be sustained by local cell proliferation as well as by an influx of cells from the peripheral blood and by recruitment from the bone marrow¹⁰.

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Maintained Function of Foreign Synapses on Hyperinnervated Skeletal Muscle Fibres of the Rat

MAMMALIAN skeletal muscle fibres normally receive input from single nerve fibres only, but will accept additional innervation from a foreign nerve if the original nerve is cut^{1,2} or anaesthetised³. We have been interested in the question of whether the extra innervation is repressed or displaced as the original innervation is re-established. Such synaptic repression has been suggested by Mark and collaborators^{4,5} for lower vertebrates and has recently attracted great interest^{6,7}.

The superficial fibular nerve, which normally innervates flexor muscles in the lower leg, was used as the foreign nerve in our experiments. It was dissected, cut peripherally, and transplanted into the proximal surface of the soleus muscle of young rats. Two weeks later the soleus was deprived of its normal nerve supply by cutting or crushing the tibial nerve in the popliteal fossa, about 15 mm from the muscle. The foreign nerve then formed synaptic contacts on soleus muscle fibres during the several weeks required for the original nerve to regenerate. Between 2 and 5 weeks after the denervation of the soleus, before regeneration of the tibial nerve was complete, we determined the extent of foreign innervation by measuring the tension developed in the soleus muscle after giving single shocks to the fibular nerve. This was done in the intact, anaesthetised animal with minimal dissection and muscle exposure. The knee joint was fixed by steel pins. A thread connected the triceps tendon to an isometric transducer and the fibular nerve was exposed for stimulation in the thigh. The deep fibular nerve branches were cut at this time to avoid interference from contraction of flexor muscles. After determining soleus twitch tension to foreign nerve stimulation and confirming early signs of re-innervation by the original nerve, wounds were stitched and the animals recovered without complication.

Terminal experiments were performed from 3 to 6 months later. First, soleus twitch tension to fibular nerve stimulation was determined in the intact leg, as above. Then the soleus muscle with transplanted and original nerves was dissected free and examined in a chamber perfused with oxygenated rat Ringer's solution. The measurement *in vitro* confirmed the extent of foreign nerve innervation of the muscle and showed the degree of re-innervation by the original nerve.

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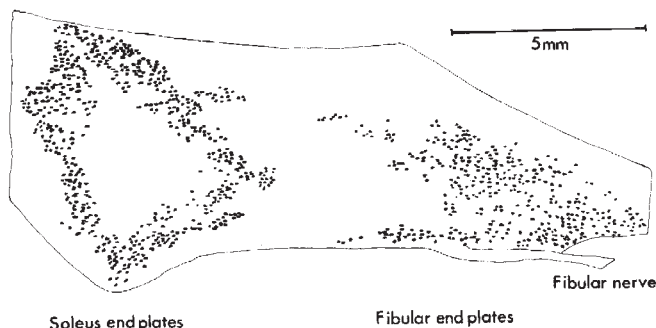


Fig. 1 Distribution of original and foreign endplates in a doubly innervated soleus muscle. The drawing outlines the proximal two-thirds of the muscle and gives the position of the end-plates as seen in a projection of a whole mount, esterase-stained preparation. The upper one-third of the muscle did not contain any foreign endplates.

In all eight rats the twitch tension *in vivo* in the soleus muscle produced by the transplanted foreign nerve during the final experiment was greater than, or in one case equal to, the one measured earlier. The average peak value was 6.5 g (range 1.4 to 12.5 g), which was approximately one-third of the tension produced by direct stimulation of the muscles. This was an average increase of 67% over the value obtained at the interim experiment (3.9 g, range 1.4 to 7 g). There was thus no suggestion of a repression of the foreign synapses, in fact, the fibular innervation increased moderately in spite of the increasing re-innervation by the original nerve supply, and the two groups of synapses remained functional for at least several months. In other experiments where the interim experiment was not performed, we have seen extensive functional foreign innervation even 9 months after re-innervation by the original nerve.

Intracellular recordings were made to study the innervation of individual muscle fibres on the dorsal surface of the muscle. In all muscles a substantial fraction of all fibres examined (12 to 38% after cutting the original nerve, 32 to 57% after crushing it) were innervated by both the foreign and the original nerves, that is, they received dual innervation. Functional foreign synapses can therefore co-exist with original innervation on single muscle fibres for prolonged periods of time. Further, extensive re-innervation by the original nerve was not prevented in these experiments.

The spatial extent of foreign innervation could be judged by sampling with a microelectrode and by visual inspection during nerve stimulation. In most muscles, fibres innervated by the foreign nerve were located only in the lateral one-third to two-thirds of the muscle (Fig. 1). Within this zone, $92 \pm 8\%$ (mean $\pm$ one s.d.) of the superficial fibres had foreign innervation. Further, the functional border, usually quite sharp, coincided closely with the anatomically defined border demonstrated by staining for ACh-esterase⁸ and by the abrupt termination of visible fibular nerve branches on the surface of the muscle. Accordingly, the presence of a substantial number of repressed foreign synapses in these muscles is unlikely.

The denervation of the original nerve supply to the muscle was usually performed in the thigh and included denervation of all the other muscles innervated by the tibial nerve. The regenerated 'original' nerve probably contained nerve fibres not originally belonging to the soleus muscle. True soleus nerve fibres might be more efficient in suppressing foreign innervation. So, in three of the eight rats we denervated the muscle by cutting only the soleus nerve between the gastrocnemius and the soleus muscle. In all these animals the soleus muscle was innervated by the foreign nerve in both the interim and the final experiments, and the situation in the soleus muscle was indistinguishable from that in the other rats.

Our findings therefore suggest that foreign synapses in adult mammalian muscle are not repressed or displaced. These results are in sharp contrast to those reported for similar experiments in fish and salamanders. Inappropriate neuromuscular synapses in these lower vertebrates are apparently repressed after muscles are re-innervated by their original nerves^{4,5}. In adult mammalian muscle, however, foreign synapses can remain functional for extended periods of time even after correct innervation has been restored.

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Behavioural Effects of *in utero* Administration of Morphine

ALTHOUGH numerous studies have been concerned with the behavioural effects of opiates, relatively little attention has been paid to the offspring of females receiving an opiate during pregnancy. The matured offspring's response to stress and to the same drug are important issues in terms of current drug abuse and potential widespread application of opiate maintenance programmes. Since morphine is readily transferred across both placental and lacteal barriers^{1,2}, we have studied the analgesic response to morphine in the offspring of mice receiving the drug during gestation and lactation.

Adult female CF 1 mice, housed in individual cages, were mated with adult CF 1 males for 1 week. After removal of the males, half the females were given a sweetened (saccharin, 0.4 g l⁻¹) morphine sulphate solution (0.5 g l⁻¹) instead of regular drinking water. Fluid intake (measured in graduated drinking tubes) in the drug group was no different from water intake in the control group. Approximately 2 weeks later, seven of the females had given birth to a total of forty-one mice, twenty in the drug group. Sex ratios were similar for the two groups. The morphine drinkers were retained on the oral drug solution for the first 2 weeks of nursing to avoid withdrawal which might upset lactation and introduce a nutritional variable. When the litters were 2 weeks old, the morphine was withdrawn and the offspring weaned, assuring that they would have had no exposure to morphine except through the maternal placenta or mammary glands. When they were 2.5 months old, the mice born to both the control and drug groups were tested for shock-elicited escape behaviour on day 1 and for morphine sensitivity on day 2.

The escape apparatus was a Plexiglas trough 10 inches long, 6 inches high, 1.5 inches wide on the floor and 4 inches wide at the top. The bottom of the trough consisted of two metal plates, 2–3 mm apart, arranged so that the animal continually bridged the two plates and received a 160 μ A foot

shock for as long as it remained in the apparatus⁹. On day 1, all mice were placed in the trough individually and the latency to jump 6 inches up onto a Plexiglass platform was recorded. If the subject did not jump up within 30 s, it was removed and a score of 30+ was recorded. The procedure was identical on day 2, except that all mice received 25 mg kg⁻¹ morphine sulphate intraperitoneally 30 min before testing.

Table 1 Response to foot shock and sensitivity to morphine

Group	Median escape latency (s)	
	Day 1	Day 2
Drug <i>in utero</i>	1.15*	8.80†
Control	3.80	30+

*, † Significantly less than control at $P < 0.01$ and 0.02 , respectively (Wilcoxon rank sum test, two tailed).

On day 1 (see Table 1) mice which had been exposed to morphine *in utero* escaped the shock with significantly lower latencies than the control offspring. On day 2, both groups had significantly ($P < 0.001$) higher latencies than on day 1, indicating an analgesic effect of the drug; but the effect was significantly less in the group whose mothers had been drinking morphine. This implies tolerance to the analgesic effect of the drug in the latter group.

The results of the day 1 experiment suggest that litters exposed to morphine *in utero* may exhibit an altered response to stress; this alteration may occur as a consequence of an increase in pain sensitivity or a hyper-reactivity to the same degree of pain. This finding may be relevant to a hypothetical role of stress in drug addiction⁴. The day 2 test for morphine sensitivity revealed tolerance to the drug. Tolerance to morphine has been observed a year after administration of a single dose to rats⁵. This study indicates that tolerance can occur in mature mice if the drug is administered through the mother during gestation and lactation. 'Personal' morphine experience with long-term consequences can therefore be similarly acquired by placental, lacteal and other routes of administration⁶. Since passive administration of morphine in adult animals can increase the probability of subsequent addiction⁷, the fact that the placental route of administration of morphine is similar to other routes seems a critical issue in opiate addiction during pregnancy.

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***In vitro* Transfer of Cell-mediated Immunity to Hepatitis B Antigen with RNA**

THE immunological basis of the chronic carrier state for hepatitis B antigen (HBAG) in man remains ill-defined. Knowledge of experimental immunity to HBAG in animal models may provide an insight into the concepts and means of terminating the human chronic carrier state, which seems to be a reservoir for transmission of type B viral hepatitis. The *in vitro* and *in vivo* measurements of cell-mediated immunity to HBAG in guinea pigs have been previously reported^{1,3}. The migration inhibition of peritoneal macrophages in the presence of HBAG is an excellent correlate of delayed hypersensitivity to HBAG in guinea pigs². We now report the *in vitro* transfer of cell-mediated immunity to non-immune cells as measured by inhibition of migration in the presence of HBAG, using RNA extracted from the lymphoid tissue of guinea pigs immunised with HBAG.

Adult albino guinea pigs of Hartley strain weighing 600–700 g were immunised by injecting 100 µg of purified HBAG subtype *ad* mixed with complete Freund's adjuvant (CFA) (ref. 2). Similarly, control animals were sensitised with CFA alone. Three weeks following immunisation, the animals were skin tested with HBAG. A strong delayed hypersensitivity reaction occurred in immunised animals, whereas no reaction to HBAG was observed in control animals. The spleen and enlarged lymph nodes of each animal were excised, and subjected to phenol extraction of RNA according to the method previously described⁴. The RNA concentration was spectrophotometrically measured and only the extracts with optical density ratios between 1.9 and 2.1 when measured at 260 nm and 280 nm wavelengths^{5,6} were employed in these studies.

The RNA extracts from animals immunised with HBAG (referred to as IRNA) and RNA from those immunised without HBAG (referred to as NRNA) were extensively dialysed against 0.3 M acetate buffer (pH 5.0) at 4° C, reprecipitated with pre-chilled 95% ethanol and dissolved in medium 199. The solutions of IRNA and NRNA at a concentration of 1 mg ml⁻¹ ($E_{1\text{cm}}^{0.1\%} = 22.0$) were used for experiments of transfer of immunity. The RNA was devoid of HBAG or anti-HBAG activity when tested by radioimmunoassay (Austria™, Abbott Laboratories, Chicago) and haemagglutination assay respectively^{7,8}.

Table 1 Transfer of Cell-mediated Immunity to HBAG Measured by Migration Inhibition of Peritoneal Exudate Cells

Treatment of normal guinea pig peritoneal exudate cells with	Migration indices
IRNA	0.10; 0.33; 0.33
NRNA	0.98; 1.00; 0.95
IRNA treated with RNase*	0.94; 0.98
NRNA treated with RNase	0.98; 0.96

The capacity of IRNA to transfer experimental *in vitro* immunity was destroyed by its treatment with RNase.

In separate reaction mixtures 0.5 ml of medium 199 containing 7×10^7 peritoneal exudate cells from a normal guinea pig was mixed with 0.5 ml solution of each IRNA and NRNA, and incubated for 30 min at 37° C. The treated cells were washed three times with Hanks balanced salt solution and packed in capillary tubes for migration inhibition assays carried out in the presence and the absence of HBAG, according to the methods previously described^{2,9,10}. For testing the effect of RNase, two 1 ml aliquots of each of the RNA preparations were incubated at 37° C for 30 min without and with 100 µg of recrystallised protease-free RNase (Calbiochem). Peritoneal cells were added to both aliquots of IRNA and NRNA, incubated at 37° C for 30 min and tested as above for migration. The results of the migration indices are depicted in Table 1.

It was clearly and repeatedly established that IRNA induced the capacity for inhibition of migration of the peritoneal

macrophages in the presence of HBAG. The NRNA failed to induce inhibition of migration in the presence of HBAG. The cells treated with NRNA, however, showed inhibition of migration in the presence of PPD which is consistent with the fact that the donors of NRNA were immunised with CFA. The migration of macrophages in the absence of HBAG was unaffected by the treatment of cells with IRNA or NRNA. It was further established that the ability of IRNA to transfer cellular immunity was destroyed by treatment with RNase. Therefore, it may be concluded that experimental transfer of cell-mediated immunity to HBAG with IRNA is a specific phenomenon induced by nondialysable RNA extracts of lymphoid organs of guinea pigs immunised against HBAG. The mechanism of this induction and the molecular characterisation of RNA involved in this transfer remain to be studied.

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Addendum: Because 'immune' RNA-mediated transfer of delayed skin reactivity to tuberculin, varidase and monilia antigens has been accomplished by injection of RNA¹¹, our results have prospects of clinical application for systemic transfer of immunity to HBAG in man.

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Origin of Unreduced Embryo Sacs in Diploid Potatoes

THERE are clear advantages in carrying out potato breeding at the diploid level, but it is likely that the expression of maximum productivity occurs at the tetraploid level¹. One way in which diploids can be brought to the tetraploid level is by 2x-4x or 4x-2x crosses. It has long been known that a large proportion of the progeny of such crosses is tetraploid^{2,3}, and it is generally recognised that unreduced gametes with the somatic chromosome number (2n) are produced by the diploid parent and these, with normal gametes (n) of the tetraploid parent, give tetraploid offspring.

Particular interest has been shown in such crosses with reports that unreduced pollen is frequently formed by the occurrence of parallel (fused) spindles at the second meiotic division, with the subsequent development of only a single cleavage furrow and dyad production^{4,7}. This is genetically equivalent to first meiotic division restitution (FDR) (ref. 8) and all loci from the centromere to the first crossover on each chromosome maintain the same constitution in the gamete as in the diploid parent. There

are, however, alternative ways in which unreduced male gametes can arise^{8,9}. These, which are genetically equivalent to second meiotic division restitution (SDR) (ref. 8), are characterised by an effective first division chromosome separation and nondisjunction of the products of the second division. The unreduced gametes produced are, in consequence, highly homozygous.

The cytological events leading to unreduced embryo sacs have not been elucidated and it is not known whether their formation can occur by FDR. We report genetic investigations which give some insight into the process.

Table 1 Ploidy and virus X reactions of progeny from 2x-4x crosses

Cross	♀ <i>Rxxr</i>	♂ <i>rx⁴</i>	Total progeny	Ploidy		4x Progeny	
				3x	4x	Resis- tant	Suscep- tible
a	H8750	Rheinhort	3	0	3	3	0
b	H8513	58.16/2	12	3	9	7	1*
c	H1890	Rheinhort	5	1	4	3	1

* One 4x seedling not tested.

Three diploid clones, H8750, H1890 and H8513, which had quite different breeding histories but were all heterozygous for gene *Rx* from *Solanum acaule*¹² (gene *Rx_{ac}*)¹³ which confers extreme resistance to virus X, were used as female parents in 2x-4x (*Rxxr-rxxrrxx*) crosses. The crosses made, and details of the progeny obtained, are given in Table 1. Ploidy level was determined by root-tip chromosome counts, and reaction to virus X by sap infection (twice) with back testing to leaves of *Gomphrena globosa*.

Table 2 Crosses made to determine genotypes of tetraploid seedlings with extreme resistance to virus X

Cross *	4x resistant progeny	×	<i>rx⁴</i>	Family
a	4628	×	62.47/20	72.27
b	4612	×	58.16/2	72.19
b	4613	×	62.47/20	72.1, 73.46, 72.21
b	4613	×	Rheinhort	72.20
b	4615	×	62.47/20	70.102
b	4615	×	Hansa	72.22, 73.42
b	4615	×	Rheinhort	72.23
b	4615	×	Grata	73.41
b	4617	×	62.47/20	72.25
b	4617	×	Hansa	73.43
c	4668	×	Record	70.9, 70.10
c	4668	×	Hydra	70.103

* 2x-4x cross: see Table 1.

As expected, most of the progeny were tetraploid (80%) and 86.7% of these proved to have extreme resistance. To ascertain whether the resistant progeny were simplex or duplex, further crosses were made with susceptible tetraploids (Table 2). The crosses involved six of the resistant seedlings from 2x-4x crosses and each was crossed with one or more different tetraploids. Particular crosses were repeated in a number of cases. The resulting families of progeny were tested for virus X reaction with the results shown in Table 3.

Only families 72.1 and 72.25 showed significant deviations from a 1:1 (or 0.87:1 with random chromatid segregation) ratio expected for simplex resistant seedlings. In the former case, further identical crosses confirmed a 1:1 ratio, and in the latter case an alternative cross with Hansa (family 73.43) gave a 1:1 ratio. In general, the data gave a better fit to a ratio based on random chromosome assortment (1:1) than random chromatid assortment (0.87:1). The results clearly show that all six resistant seedlings were simplex, and that 2n gametes involved in the crosses producing these seedlings had the same genetic constitution as the parents with respect to this locus.

SDR, in the absence of crossover between the centromere and the *Rx* locus, would give gametes homozygous

at this locus. Tetraploid progeny might be expected to segregate 1:1 (resistant:susceptible) with resistant progeny duplex for *Rx*. These duplex progeny would segregate 5:1 in subsequent crosses with susceptible tetraploids. The evidence suggests that unreduced embryo-sac formation was not by SDR since it is unlikely that all the resistant progeny tested had resulted from crossing over between the centro-

Table 3 Segregation in crosses made to determine genotypes of tetraploid seedlings with extreme resistance to virus X

Family *	Progeny		Probability of a larger value of χ^2	
	Resistant	Susceptible	1:1†	0.87:1‡
72.27	9	11	0.75-0.50	0.90-0.75
72.19	56	45	0.25-0.10	0.10-0.05
72.1	26	10	<0.005	<0.005
73.46	22	24	0.75-0.50	0.90-0.75
72.21	14	12	0.75-0.50	0.50-0.25
72.20	10	9	0.75-0.50	0.75-0.50
70.102	26	19	0.50-0.25	0.25-0.10
72.22	7	12	0.25-0.10	0.50-0.25
73.42	21	16	0.50-0.25	0.25-0.10
72.23	29	26	0.75-0.50	0.50-0.25
73.41	81	77	0.75-0.50	0.25-0.10
72.25	23	11	0.05-0.025	<0.005
73.43	27	28	0.90-0.75	0.75-0.50
70.9	52	52	1.00	0.50-0.25
70.10	57	71	0.25-0.10	0.75-0.50
70.103	8	7	0.90-0.75	0.75-0.50
	468	430	0.25-0.10	<0.005

* See Table 2.

† Rxx^3-rx^4 assuming random chromosome assortment.

‡ Rxx^3-rx^4 assuming random chromatid assortment.

mere and the *Rx* locus. The 13:2 (resistant:susceptible) ratio is indicative of FDR which results in simplex resistant tetraploids. Crossing over could account for the two susceptible tetraploids obtained since $rrxx$, Rxx and $RxRx$ (1:2:1) gametes should originate in this way. That duplex resistant progeny formed by the involvement of $RxRx$ gametes were not found was probably due to the small number of resistant progeny examined. The alternative theory of Koopmans and Van der Burg¹⁴, that mitotic doubling of the reduced chromosome number in the egg nucleus of a diploid occurs when pollen of a tetraploid enters the embryo sac, can be excluded since tetraploid progeny would all be nulliplex or duplex.

The potential importance in breeding of the use of unreduced gametes formed by FDR lies in the possibility offered of preserving beneficial intra-locus and inter-locus interactions present in the 2x genotype and transferring them, more or less intact, to the 4x progeny⁶. The use of such intact chromosome sets should, Chase has suggested¹, maximise heterotic potential. Striking heterotic responses and an unusually high degree of within-family uniformity in field characteristics has been observed in 4x-2x progenies⁴⁻⁶ but not in 2x-4x^{4,10,11}, and it has been suggested that this reflects fundamental differences between the modes of unreduced pollen and embryo-sac formation. Our own field observations do not suggest fundamental differences in the progenies of 4x-2x and 2x-4x crosses, and this work suggests that unreduced embryo sacs can arise via FDR. In view of the potential value of such inter-ploidy crosses further investigations to determine the relative frequencies of FDR and SDR in the production of both unreduced pollen and embryo sacs would be of the utmost interest.

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Absence of Messenger RNA for Beta Globin Chain in β^0 -Thalassaemia

THE β -thalassaemias are a heterogeneous group of hereditary haematological disorders in which there is absent or decreased synthesis of the β globin chain of normal human adult haemoglobin, HbA ($\alpha_2\beta_2$)¹. When β chain synthesis is totally absent, the condition can be referred to as β^0 -thalassaemia, in contrast to β^+ -thalassaemia in which some β chain synthesis occurs. The genetics and manifestations of β^0 -thalassaemia are rather varied. In the heterozygote, there may be either increased levels of the minor haemoglobin, HbA₂ ($\alpha_2\delta_2$) ('A₂-thalassaemia'), or normal levels of HbA₂ with elevated levels of foetal haemoglobin, HbF ($\alpha_2\gamma_2$), (' $\delta\beta$ -thalassaemia' or 'F-thalassaemia'). Homozygous β^0 -thalassaemia of the A₂ variety is clinically severe, and has been described mainly in the Ferrara region of northern Italy and in Thailand, although it is found sporadically in other racial groups; the red cells of these individuals contain mainly HbF with variable amounts of HbA₂, but no HbA. Homozygous $\delta\beta$ -thalassaemia is extremely rare, having been described in only eight individuals from four different families¹; it is associated with only mild symptoms; the red cells of these individuals contain 100% HbF, and there is total absence of both HbA and HbA₂.

In the usual form of homozygous thalassaemia (β^+ -thalassaemia of the A₂ variety), the decreased synthesis of β chain has been shown to be associated with a deficiency of functional mRNA for β globin chains: mRNA isolated from β -thalassaemic reticulocytes (and marrow cells), when translated in heterologous cell-free systems, programmes the synthesis of much less β than α chain of HbA²⁻⁷. Furthermore, the β chain mRNA has been shown to be truly deficient (quantitatively) in these patients: hybridisation of thalassaemic mRNA with synthetic DNA (cDNA) complementary to α or β globin mRNA reveals the presence of much less β mRNA than α mRNA^{8,9}. In β^+ -thalassaemia, therefore, there is no evidence for the presence of normal amounts of a functionally and/or structurally abnormal β chain mRNA unable to participate in protein synthesis. It has been proposed that the β^0 -thalassaemia of the Ferrara region may have a different molecular basis: experiments have been performed which suggest the presence, in this syndrome, of substantial amounts of β chain mRNA. When ribosomes from reticulocytes of patients with homozygous Ferrara thalassaemia are incubated in a cell-free system in the presence of supernatant fraction of non-thalassaemic reticulocytes from persons with HbA or HbS, some β^A (but no β^S) chain is apparently synthesised^{10,11}.

We have now examined by the method of DNA-RNA reannealing or hybridisation, mRNA from two unrelated patients with homozygous β^0 -thalassaemia of the A₂ variety,

and pooled reticulocyte RNA from three siblings with homozygous $\delta\beta$ -thalassaemia. The patients were of southern Italian ancestry and not from Ferrara; the $\delta\beta$ -thalassaemics were Sicilian and have been previously reported¹². Our results indicate a virtual absence of β chain mRNA in reticulocytes of these patients.

A major difficulty in these studies resulted from the presence, in β -thalassaemic red cells, of mRNA for the γ chain of HbF, and the cross homology which evidently must exist in the mRNA sequence coding for the human γ and β globin chains, which differ only in thirty-nine of 146 amino acids.

Hybridisation of globin mRNA to cDNA under non-stringent conditions, or hybridisation of human mRNA to cDNA probes synthesised from non-human globin mRNA as template, can lead to an incorrect assessment of the true amount of β chain mRNA present in a given preparation because of the formation of γ mRNA- β cDNA hybrids^{8,9}. We have shown, however, that hybridisation of human globin mRNA with a cDNA probe specific for human β chain mRNA, under stringent conditions, permits the assay of the amount of β chain mRNA present in a given RNA sample without interference from the γ chain mRNA present in that sample⁸. The human β cDNA probe was synthesised from α -thalassaemia mRNA which was shown to contain α : β sequences in a ratio of 1:6⁸; and the conditions required to eliminate γ mRNA- β cDNA hybrid formation was to increase hybridisation temperature from 65° C to 78° C⁸.

Our initial experiment involved the use of a labelled cDNA probe, synthesised from total human (non-thalassaemic) globin mRNA. This probe apparently consisted of α and β chain sequences in a ratio of approximately 1 to 1. Hybridisation was performed for 40 h at 78° C in 0.2 M sodium phosphate buffer, pH 6.8, and 0.5% SDS. The amount (%) of the input cDNA which formed a hybrid, with the mRNA,

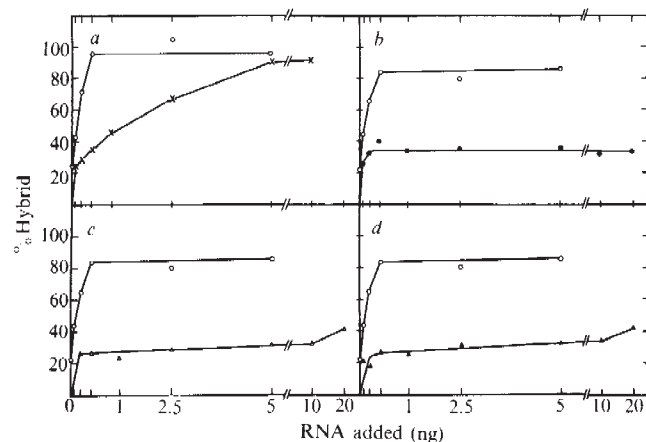


Fig. 1 Hybridisation of human reticulocyte RNA to total human globin cDNA. The cDNA was prepared as previously described⁸, but in the presence of ^3H -dCTP, 25.5 Ci mmol⁻¹, and non-thalassaemic human globin mRNA. The cDNA input was 220 to 300 TCA-precipitable c.p.m., per reaction; the blanks, after digestion with S1 nuclease, contained 20 to 30 c.p.m. The globin mRNA was prepared from total reticulocyte lysates as previously described³ and, except for the thalassaemic mRNA in panels *b* and *d*, consisted of the RNA sedimenting between 4S and 18S after sucrose gradient fractionation^{3,6}. The thalassaemic RNA in panel *b* consisted of total unfractionated RNA, and in panel *d*, the gradient fractionated RNA sedimenting faster than 4S and including the 28S RNA. The indicated amount of RNA refers to the amount of added 10S globin mRNA as determined by acrylamide gel electrophoresis of the RNA samples or by assuming 10S RNA to be 1% of total cellular RNA. Hybridisation was for 40 h at 78°–80° C, and % hybridisation was determined as previously described⁸. *a*, $\circ$ — $\circ$, non-thalassaemic human globin mRNA. *a*, $\times$ — $\times$, β^+ -thalassaemic mRNA (same patient as in ref. 8). *b*, $\bullet$ — $\bullet$, $\delta\beta$ -thalassaemia RNA. *c*, $\triangle$ — $\triangle$, β^0 -thalassaemia (A_2 type): patient 1. *d*, $\blacktriangle$ — $\blacktriangle$, β^0 -thalassaemia (A_2 type): patient 2.

was then determined by the digestion of the sample with *Aspergillus* S1 nuclease as previously described⁸.

The results are shown in Fig. 1, where percentage of hybrid formation is plotted on a linear scale, with respect to increasing amounts of added 10S RNA to each sample. RNA derived from reticulocytes of a non-thalassaemic patient showed a saturation curve with an initial saturation value of 95%, achieved at a very low RNA input (0.5 ng) (Fig. 1*a*). This result indicates that both α and β chain sequences were present in the sample in reasonably high concentration. A sample of RNA derived from reticulocytes of a patient suffering from β^+ -thalassaemia also reached a final saturation of 90–95% but two transitions are observed in the saturation curve. The initial slope, which reached a value of approximately 40–45% hybridisation, probably represents mainly hybridisation of the α chain sequences present in the sample, and it was achieved at a relatively low RNA input (0.5–1.0 ng) [Fig. 1*a*]. The second transition, then, would represent hybridisation of the reduced amount of β chain mRNA present in this sample: 90–95% hybridisation was finally achieved at a RNA input of 5–10 ng.

The hybridisation of RNA derived from patients with homozygous $\delta\beta$ -thalassaemia (Fig. 1*b*) showed a first transition identical to the transition shown by the β^+ -thalassaemic patient: approximately half as much hybridisation as the control (non-thalassaemic RNA) was achieved with 0.5 ng of RNA. No second transition was, however, observed: even after the addition of forty times more RNA (20 ng), there was roughly only half as much hybridisation as achieved by the control RNA, at saturation. This result strongly suggests that some of the sequences present in the normal reticulocytes were absent in the reticulocytes derived from the patients with $\delta\beta$ -thalassaemia. RNA derived from reticulocytes of two patients with homozygous β^0 -thalassaemia, of the A_2 variety, was also assayed (Fig. 1*c* and *d*). The saturation curves are similar to the curve observed for the RNA derived from the $\delta\beta$ -thalassaemia reticulocyte RNA except that in both cases there is a suggestion of the start of a second transition at the highest RNA input (20 ng). These results also indicate that some mRNA sequences present in normal reticulocyte RNA were absent or present in extremely low amounts in the reticulocyte RNA of these patients with β^0 -thalassaemia.

To assay specifically for β chain mRNA in the RNA samples of these patients, experiments similar to those shown in Fig. 1 were performed, but using a probe specific for human β chain mRNA:cDNA transcribed from α -thalassaemia mRNA⁸. Hybridisation was again performed under the stringent conditions (78–80° C) which eliminated cross-hybridisation between γ and β chain sequences. The results of these experiments are shown in Fig. 2. The non-thalassaemic and β^+ -thalassaemic RNA (Fig. 2*a*) yielded saturation curves similar to those obtained in Fig. 1*a*, except that the β^+ -thalassaemic RNA showed much less hybridisation at low RNA input and achieves saturation only at a higher RNA input than previously: the deficiency of β mRNA is even more obvious than in Fig. 1*a*. In Fig. 2*b* it can be clearly seen that β chain sequences were totally absent in the RNA derived from reticulocytes of the patients suffering from homozygous $\delta\beta$ -thalassaemia: at all mRNA concentrations tested ranging from 0.25 to 100 ng, there was only a baseline level of hybridisation (15%), consistent with the estimated 15% 'contamination' of the β mRNA (α -thalassaemia mRNA) with α mRNA sequences⁸. Hybridisation of mRNA derived from reticulocytes of the two patients with β^0 -thalassaemia of the A_2 variety also shows virtual absence of β chain mRNA sequences (Fig. 2*c* and *d*).

A small amount of hybridisation above the 15% baseline level was observed at very high RNA inputs of 50 to 100 ng in A_2 - β^0 -thalassaemia (Fig. 2*c* and *d*). We suggest that this hybridisation may represent hybridisation of trace amounts of δ chain mRNA sequences (of HbA₂) with the β chain

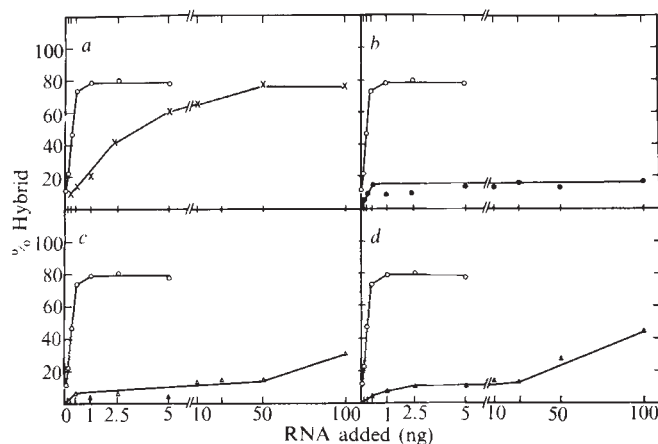


Fig. 2 Hybridisation of human reticulocyte RNA to human β cDNA. The cDNA was synthesised in the presence of ^3H -dCTP (25.5 Ci mmol $^{-1}$) and human β -chain-specific (α -thalassaemic) globin mRNA⁸. Hybridisation was for 40 h at 78–80° C. The input cDNA was 200–500 c.p.m. and the digested blank had 20–30 c.p.m. The RNAs and their symbols are the same as in Fig. 1.

cDNA probe; the quantity involved was less than 0.5% of the RNA which hybridised in Fig. 1c and d. The homology between δ and β chains, which differ in only ten of 146 amino acids, is greater than that between γ and β chains and, therefore, cross hybridisation would be expected even under the stringent conditions used. It is not possible to obtain δ chain mRNA in sufficient amounts and purity for the synthesis of δ specific cDNA probes to test this hypothesis. Although it cannot be conclusively established that the small amount of hybridisation observed at high RNA inputs represented δ chain mRNA rather than trace amounts of true β chain mRNA, it is nonetheless clear that very little (if any) β chain mRNA was present in the reticulocyte RNA of our patients with β^0 -thalassaemia. Certainly, these patients of southern Italian ancestry did not have the defect which has been proposed for the Ferrara type of β^0 -thalassaemia. Finally, it should be noted that all the RNA samples tested were derived from total reticulocyte lysates and not from polysomes: our experiments, therefore, ruled out the existence in these thalassaemic reticulocytes of β chain mRNA which is unable to enter polysomes and participate in protein synthesis. Furthermore, the use of unfractionated RNA and RNA which includes all of the RNA between 4S and 28S, ruled out the unlikely existence of a high molecular weight abnormal mRNA which would otherwise be missed by using only RNA from the 9–10S region of the sucrose gradient.

In conclusion, these results suggest that a feasible hypothesis to explain the molecular defect, in certain forms of β^0 -thalassaemia, is a deletion of the DNA sequences coding for the β chain mRNA (and, in addition, for δ chain mRNA in $\delta\beta$ -thalassaemia). The ability to hybridise β chain cDNA sequences, specifically, in the presence of vast excesses of cellular DNA from patients with β^0 -thalassaemia, will permit the testing of this hypothesis.

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Renal Concentration of Citrate as a Negative Modulator of Diuretic Response to Mercurials

In several mammalian species, including man, the potency of organomercurial diuretics depends on the acid-base status. Acidifying salts (for example, ammonium chloride) markedly enhance the diuretic response to mersalyl¹. To account for this, Weiner *et al.*² proposed that (a) hydrogen ions facilitate the cleavage of the carbon-mercury bond of organomercurials and (b) the diuretic response is elicited by the interaction of mercuric ions with renal diuretic receptors. The authors based their conclusions on the excellent correlation between the liberation of mercuric ions from a given organomercurial under acidotic conditions *in vitro* and its diuretic potency in dogs *in vivo*. Recently we reported that rapid alkalisation of urine brought about by treatment of dogs with acetazolamide decreases the diuretic response to inorganic mercury complexed with cysteine³. This observation led us to postulate that alterations in acid-base balance not only modify the rate of cleavage of the carbon-mercury bond in organomercurials but also must control the diuretic response to mercuric ions. Our findings are compatible with the following hypothetical chain of events. Alkalosis increases and acidosis decreases the renal concentrations of an en-

Table 1 Urinary pH and tissue citrate concentrations in dogs

Before or after treatment	Acid	Treatment Base Urinary pH	NaFAC
Before	6.25	7.05	6.91
After	5.35	7.89	6.99
Urinary excretion of citrate ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)			
Before	0.0024	0.0017	0.0025
After	0.0030	0.068	0.47
Plasma citrate ($\mu\text{mol ml}^{-1}$)			
Before	0.120	0.152	0.115
After	0.052	0.152	0.249
Citrate in renal cortex ($\mu\text{mol g}^{-1}$ fresh weight)			
After	0.048	0.131	2.61
Citrate in renal medulla ($\mu\text{mol g}^{-1}$ fresh weight)			
After	0.069	0.180	0.682

The animals were killed after treatment for 2 h with hydrochloric acid or sodium hydroxide (priming dose of $1,000 \mu\text{mol kg}^{-1}$ intravenously and sustaining infusion of $1,000 \mu\text{mol kg}^{-1} \text{h}^{-1}$) or with sodium fluoroacetate (Na-FAC, 0.5 mg kg^{-1} intravenously). The values represent averages for two dogs in each treatment group.

ogenous metabolite which is capable of complexing mercury. In the kidneys the partition of mercuric ions between the diuretic receptor and the hypothetical ligand will then depend on the relative concentration of these two substances and their relative affinity for mercury. Specifically, the ratio of mercury bound to these two sites can be described by the following expression

$$\frac{[\text{Hg-L}]}{[\text{Hg-R}]} \propto \frac{[\text{L}] \cdot K_L^{\text{Hg}}}{[\text{R}] \cdot K_R^{\text{Hg}}}$$

where brackets denote the renal concentration, L the hypothetical ligand, R the diuretic receptor, Hg-L and Hg-R the corresponding complexes with mercury, and K^{Hg} the effective stability of mercury complexes with the compound denoted in the subscript. A change in any one of the four factors on the right side of the expression will alter the partition of mercury between these two binding sites. If the concentration of the ligand increases in alkalosis, more mercury will be sequestered by the ligand and, as a consequence, a smaller number of mercuric ions would be free to react with renal diuretic receptors. The predicted result will be a

decreased diuretic potency of inorganic mercury. Under acidotic conditions when the renal level of the ligand is low, the binding of mercury to the diuretic receptor would not be opposed to the same extent. Therefore the full diuretic response will ensue. The hypothesis does not require that changes in acid-base status alter either the total concentration of mercury in renal tissue or the rate of urinary excretion of mercury. In any case a large fraction of renal mercury is bound to non-specific sulphhydryl groups. Many sulphhydryl groups of proteins may serve as scavengers for mercury and thus do not participate in the diuretic response^{1,4}.

Citrate anion most fully meets all the requirements of the hypothetical ligand. First, citrate forms stable chelates with a host of divalent cations⁵. The stability of the mercury-citrate chelate has not been determined. The absolute value of the stability constant of the mercury-citrate complex is, however, not required to test the hypothesis. The main postulate—that a change in citrate concentration produces a shift in the intrarenal distribution of mercury—holds true independent of the affinities of the diuretic receptor and citrate for mercury. Even if the stabilities of mercury complexes with the diuretic receptor and with citrate were known and if the former were found to be 100 times greater than the latter, citrate may still compete effectively with the receptor for mercury if its concentration is about 100 times greater than that of the receptor. In regard to the second postulate, changes in acid-base status produce the requisite changes in renal level of citrate. In the kidneys of rats, citrate levels are elevated under alkalotic and decreased under acidotic conditions when compared to control values⁶⁻⁸.

To test our hypothesis we chose conditions which would alter the renal concentration of citrate with or without inducing concomitant changes in acid-base status. Two series of experiments were performed on anaesthetised dogs of either sex. Experimental procedures were identical to those described elsewhere⁹. The first series of experiments was intended to determine whether the citrate concentrations in the kidneys are altered in the desired direction in dogs infused with hydrochloric acid or sodium hydroxide for 2 h, or injected with sodium fluoroacetate. Citrate in urine, kidneys and plasma was determined by the method of McArdle¹⁰. The data of Table 1 show that the conditions are met and, in particular, that fluoroacetate elevates renal concentrations of citrate without changing urinary pH.

In the second series of experiments, dogs were treated as in the first series. At the end of the 2 h pretreatment period

Table 2 Glomerular filtration rate and the urinary excretion of electrolytes in dogs

	Acid	Acid and uranyl	Base	NaFAC
GFR ($\text{ml kg}^{-1} \text{min}^{-1}$)				
Before Hg	3.88 $\pm$ 0.50	4.01 $\pm$ 0.16	3.60 $\pm$ 0.61	3.91 $\pm$ 0.14
Change	-0.50 $\pm$ 0.25	-0.95 $\pm$ 0.10	-1.04 $\pm$ 0.19	-1.36 $\pm$ 0.10
Na excretion ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)				
Before Hg	25.4 $\pm$ 7.4	18.7 $\pm$ 4.3	26.8 $\pm$ 4.1	39.2 $\pm$ 6.8
Change	71.3 $\pm$ 10.5	50.3 $\pm$ 2.3	13.0 $\pm$ 11.3	2.5 $\pm$ 9.9
Chloride excretion ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)				
Before Hg	26.3 $\pm$ 7.1	20.0 $\pm$ 4.1	14.3 $\pm$ 2.1	35.5 $\pm$ 6.8
Change	63.8 $\pm$ 12.0	51.2 $\pm$ 3.5	11.5 $\pm$ 9.9	2.5 $\pm$ 8.3
K excretion ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)				
Before Hg	6.01 $\pm$ 0.40	4.11 $\pm$ 0.54	5.57 $\pm$ 0.72	7.86 $\pm$ 0.66
Change	0.81 $\pm$ 0.08	0.15 $\pm$ 0.36	-1.85 $\pm$ 0.70	-4.85 $\pm$ 0.99

Dogs were pretreated with hydrochloric acid, sodium hydroxide or sodium fluoroacetate as described in Table 1, and subsequently injected with mercury-cysteine complex [$5 \mu\text{mol Hg}(\text{NO}_3)_2 + 25 \mu\text{mol cysteine kg}^{-1}$ intravenously]. Uranyl benzoate ($3.5 \mu\text{mol kg}^{-1}$ intravenously) was injected just before mercury in one group of dogs pretreated with hydrochloric acid. GFR was measured as the clearance of inulin. Changes were during the second hour after the injection of mercury. Four dogs in each treatment group. Data are given as mean $\pm$ s.e.

* $P < 0.05$. † $P < 0.01$; that the changes differ from zero.

(see above) mercury-cysteine complex was injected [5 μmol $\text{Hg}(\text{NO}_3)_2$ + 25 μmol cysteine per kg body weight intravenously]. To produce a decrease in the glomerular filtration rate (GFR) comparable with that expected in the two other groups, uranyl benzoate (3.5 μmol kg intravenously) was administered to groups of acidotic dogs just before the injection of mercury. Except for the effect on GFR, uranyl does not interfere with mercurial diuresis¹¹. The excretion of electrolytes in urine was followed for 2 additional hours. During the second hour a marked diuretic response to mercury-cysteine complex was observed in all acidotic dogs but not in the four alkalotic animals or in those treated with fluoroacetate (Table 2). There was a slight decrease in GFR in dogs pretreated with acid alone and a larger decrease in acidotic dogs treated with uranyl benzoate, in alkalotic dogs, and in fluoroacetate pretreated dogs. It is clear, therefore that a drop in GFR though reducing the maximal diuretic response to mercury cannot account for the total absence of diuresis in alkalotic dogs or in those pretreated with fluoroacetate. The results are thus in agreement with and furnish experimental support for our hypothesis. The following additional observations were made. (a) Urinary excretion of radiomercury, administered along with mercury-cysteine complex, was similar in all four groups. (b) The corollary to the hypothesis, namely, that fluoroacetate should not interfere with the diuretic response to nonmercurial diuretics was demonstrable. The diuretic response to ethacrynic acid (5 mg kg^{-1} intravenously; two dogs) and furosemide (10 mg kg^{-1} intravenously; two dogs) was not inhibited by the pretreatment with fluoroacetate. In these dogs the increment in the urinary excretion of sodium amounted to 40 to 50 μmol kg^{-1} min^{-1} . The diuretic response to ethacrynic acid is especially noteworthy since mercurials and ethacrynic acid may have the same mechanism of action at the cellular level⁹. If renal citrate prevents mercurial diuresis by chelating mercuric ions then the observed diuretic response to ethacrynic acid agrees with our hypothesis since a corresponding reaction between citrate and the anion of ethacrynic acid cannot occur. (c) Renal concentrations of citrate in normal rats, as reported by Crawford⁶ and others^{7,8}, are higher than in alkalotic dogs in our experiments. This fact is in accordance with the known low potency of mercurial diuretics in rats. Our preliminary study in this species, in addition to confirming the older data, showed a four-fold increase of citrate concentrations in the kidneys, but not in the liver, of rats treated with mercury-cysteine complex. Mercurial diuretics may, therefore, be 'self-inhibitory' in this species.

Although all these findings support our hypothesis, the possibility that increased renal concentrations of citrate alter the response to mercurial diuretics in some way other than by chelation of mercuric ions cannot be excluded at the present time.

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Autoradiography of Biological Tissues in the Scanning Electron Microscope

AUTORADIOGRAPHY combined with light or transmission electron microscopy has become a very valuable tool in biological research^{1,2}. Light microscope (LM) autoradiographic resolution is, however, limited by the resolving power of the optical system. A ten to twenty times improvement in autoradiographic resolution (up to 50 to 70 nm) can be obtained in the transmission electron microscope (TEM) but a number of physical factors limit the system^{1,2} including the size and the sensitivity of the photographic emulsion silver halide crystal and the grain-size. Further, ultrathin TEM sections limit detection of radioactive material in labelled specimens by the very small fraction of total incorporated radioactivity present in such sections. This leads to a practical limitation of TEM autoradiography for, in addition to the already time-consuming preparation of ultrathin sections, high levels of radioactive labelling and long exposure times (about ten times that of LM autoradiography) must in general be used to compensate for the low efficiency of the procedure.

It is of interest, therefore, to consider the possibility of combining autoradiography with scanning electron microscopy (SEM)³ as the resolution of the SEM (7 to 20 nm), although inferior to that of the TEM, is within the range attained in TEM autoradiography. A number of advantages are suggested by the use of such a system including the ease and rapidity of specimen preparation, the larger sample areas and the possible improvement of autoradiographic efficiency using smaller crystal emulsions and finer compact grains—detection of developed grains being helped by the depth of field and analytical facilities of the SEM.

In this preliminary investigation on the biological application of SEM autoradiography bladder explants from 6-month-old C57^{B1}/Icr mice were cultured *in vitro*⁴ for 4 d on medium containing ³H-thymidine (1 μCi ml^{-1} , 5 Ci mmol^{-1}) and 4 h before fixation colchicine (0.1 mg ml^{-1}) was added to the medium. The tissues were fixed in Bouin's fluid, embedded in paraffin wax and 5 μ thick serial sections prepared. These were mounted on carbon-coated⁵ and on uncoated 13 mm Chance coverslips. Control non-radioactive explants were processed in parallel with the radioactive tissues.

To facilitate subsequent manipulation of the coverslips, these were provisionally mounted with DPX, section-side uppermost, on glass slides. The sections were dipped in a 1:1 Ilford L4 nuclear track emulsion and exposed for 1 week at 4° C (ref. 6). The autoradiographs were developed with Kodak D19 for 5 min at 18° C and fixed for 10 min in Ilford Hypam. These were either left unstained and air-dried for scanning electron microscopy or post-stained with Mayers' haematoxylin-eosin stain and mounted for light microscope observations. To determine if the surface detail of the emulsion-covered sections could be enhanced for scanning electron microscopy the gelatin of the emulsion was removed in a number of sections by treatment with 0.05 N sodium hydroxide for 20 min at 18° C (ref. 7). Specimens mounted on carbon-coated coverslips were viewed initially in the SEM without any metal coating. Subsequently, an

approximately 20 nm electrically conducting layer of aluminium or gold was applied to both carbon-coated and uncoated coverslips. The specimens were examined with a Cambridge Scientific Instruments Stereoscan Mk IIa scanning electron microscope using an accelerating voltage of 30 kV and a specimen tilt angle of 45° unless otherwise noted. With an Ortec Si(Li) solid state energy dispersive detector coupled with a Northern Scientific Econ II multi-channel analyser, X-ray elemental and distribution analyses of silver were mapped using the $L\alpha$ line. The X-ray analyses were performed at an accelerating voltage of 20 kV, beam current of 120 μ A, and a filament current of 2.5 A. Lens settings were at 0.42 A (top), 0.37 A (middle), and 0.60 A (final lens). X-ray count times varied from 3,000 to 5,000 s.

Light microscope observations showed concentrations of black silver grains over the cell nucleus, the radioactive material being present mainly in the epithelial cells of the bladder cultures with very little uptake of label occurring in the stromal tissues. Specificity of labelling was demonstrated by a comparison with the control, non-radioactive bladder sections which showed only a very low random background distribution of grains. A specific distribution of grain-like structures, similar to that seen under the light microscope, was found in the scanning electron autoradiographs (Fig. 1a). This distribution could be directly correlated with the light microscope autoradiograph by light microscope observations of the SEM specimen (Fig. 1b). Because of their higher back-scattering coefficient, the grain structures were easily identified in the uncoated sections on carbon-coated glass (Fig. 1a). Although at low magnifications charging problems were unimportant, it was necessary to metal coat the specimens to make detailed observations of the grain structures at higher magnifications. The metal coating had the effect of reducing the difference in back-scattering coefficients between the silver and the matrix. Structural details of the section were found to be enhanced following sodium hydroxide treatment (Fig. 2).

Irregular, ribbon-like filaments, thickened at discrete points along the surface extended from crystalline structures which, in general, demonstrated a cubic shape (Fig. 3). This morphological picture correlates closely with published photomicrographs in transmission electron microscope studies on silver filament formation⁸⁻¹⁰ and the grain structures observed in the scanning electron micrographs may be interpreted, therefore, as developed grains consisting of metallic silver ribbons extending from cubic silver halide crystals. It was sometimes difficult to see the characteristic filamentous form of the D19 developed L4 emulsion grains¹¹, but structural visualisation was markedly improved by alteration of the tilt angle of observation from 45° through 15° or 0°. The distribution of silver grains was

easily determined using back-scattered electron imaging¹² in the scanning electron microscope. This was found to provide a system analogous to darkfield illumination in LM autoradiography¹ in that the silver grains, because of their higher atomic number, have a back-scattering coefficient more than four times that of the tissue material¹³, and appeared as bright structures on a dark background (Fig. 4a and b). The brightness and structural definition of the silver grains were dependent on the angle of tilt of the specimen to the incident electron beam (Fig. 4a and b) and the highly electron reflective silver halide crystals could only be readily distinguished from the less electron-reflective silver filaments at low angles

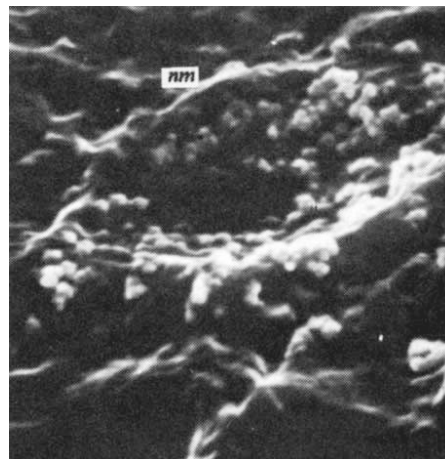


Fig. 2 SEM autoradiograph of sodium hydroxide treated bladder section (Au coated) showing grain concentration in nucleus and resolution of nuclear membranes (nm) (45° tilt) ($\times 11,400$).

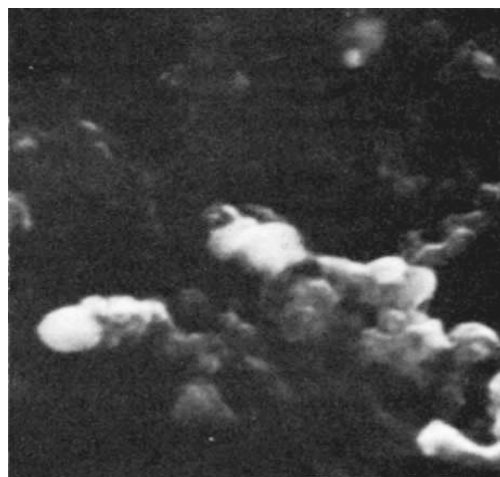


Fig. 3 SEM micrograph of silver grains showing irregular ribbon-like filaments extending from highly electron-reflective cubic Ag halide crystals (15° tilt) ($\times 12,000$).

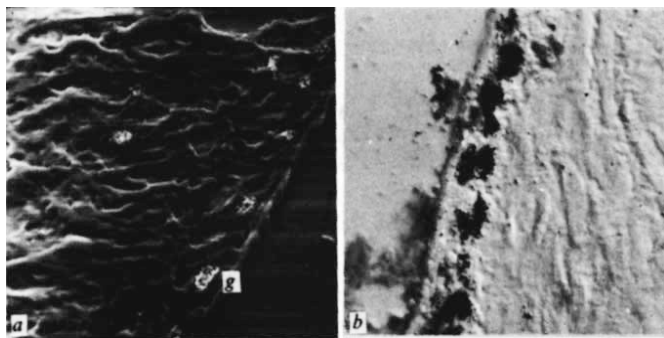


Fig. 1 Autoradiographs of bladder showing concentrations of silver grains over nuclei of cells. a, Uncoated SEM preparation mounted on carbon-coated coverslip. Grains, g, are easily identified by their higher back-scattering coefficient (45° tilt) ($\times 213$); b, light micrograph of (a). ($\times 135$).

of tilt (0° to 15°). The background grains, found randomly scattered over the sections and glass coverslips were noticeably smaller than the grains produced over sites of radioactivity and corresponded to previous LM observations¹.

Further evidence of the nature of the grain structures was provided by X-ray elemental analysis for silver over labelled and unlabelled areas of the tissue and by distribution mapping of the Ag X rays. Spot analyses on individual grains and small area X-ray analyses over labelled bladder epithelium showed well-defined Ag $L\alpha$ peaks with peak-to-background

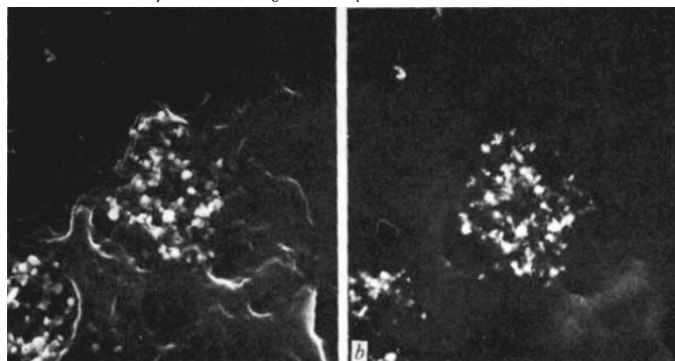


Fig. 4 SEM autoradiograph of bladder epithelial cells (Au coated). *a*, Secondary emissive; *b*, back-scattered electron image analogous with LM darkfield illumination¹ (15° tilt) ($\times 2,130$).

ratios of 4:1 and 2:1 respectively, while only a trace of silver was found over unlabelled cells. X-ray distribution mapping of silver using the $L\alpha$ line showed silver localised in regions of grain concentrations.

Our observations show that autoradiography combined with scanning electron microscopy should provide a technique enabling rapid autoradiographic localisation of various compounds which can be correlated with fine structural detail. The system promises to be of particular value combined with automated image analysis procedures¹⁴ for quantitative autoradiographic data retrieval.

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Lengthening of Illumination Period is a Factor in Averting Diapause

DURING studies of wing length and diapause of *Gerris odontogaster* (Zett.) (Heteroptera, Gerridae), I claimed¹ an intimate correlation between the micropterous and non-diapause conditions and the macropterous and diapause conditions respectively, in pre-overwintering populations. I also

summarised the general determination of wing lengths and diapause (Fig. 1), and later a macropter with fully developed eggs was collected from a dimorphic population.

The effect of stationary daylengths, however, was omitted from the figure and although I demonstrated¹ that in a rhythm of 18 h at about 1,000 lux: 6 h at about 80 lux (instead of a planned dark period) both macropter and micropter imago emerged, the reproductive stage was left implicit in the text. Therefore, my conclusion¹ that a lengthening day averts diapause, has been criticised because of lack of evidence in stationary light conditions².

To settle this problem, results of six partly unpublished experiments are now given here (Fig. 2). In experiment *E* some parentals collected on September 14 were first kept at room temperature (about 24° C) in natural photoperiod. No eggs were produced and the individuals became inactive. They were transferred on October 10, to a light:dark photoperiod of 24:0. The first copula was seen on October 30. The rest of the parental batch was taken straight to continuous illumination on September 15. First larvae emerged on October 6, that is, egg laying had begun in September. Therefore the breaking of diapause seems

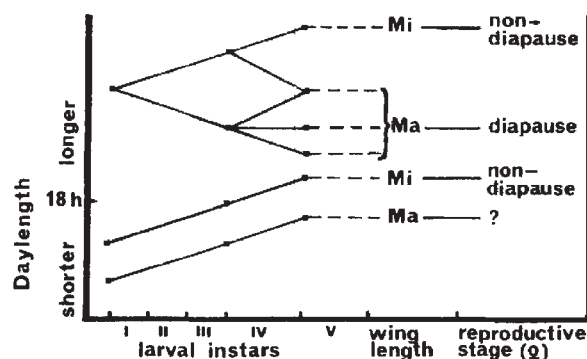


Fig. 1 Determination of wing length and diapause in *Gerris odontogaster* according to ref. 1. The last critical stage is the fourth instar. Mi, Micropter imago; Ma, macropter imago.

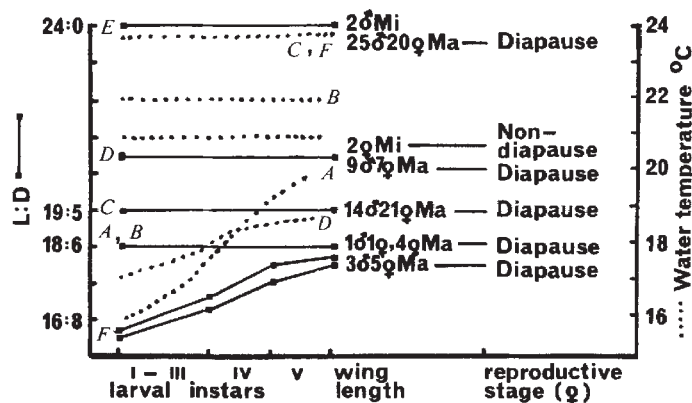


Fig. 2 Reproductive diapause in *Gerris odontogaster* as a response to long, stationary illumination or short, lengthening day rhythm. Light equipment as in ref. 1. The reproductive stage (egg laying) was checked by keeping the adult offspring in cultures from 4 to over 20 weeks and sometimes by dissecting them later. The only offspring to lay eggs were the micropters (*D*), one in the age of ≤ 15 d, and the other of ≤ 11 d. Parentals: *A*, *F*₁ generation macropter from ref. 1; *B*, *C*, *E*, *F*, from the Finnish uniform grid 664:28 and, *D*, 671:23. In *C*, some few individuals developed through their fifth instar in a long, shortening day rhythm. In experiment *D*, the L:D ratio was 18 h (1,000 lux): 6 h (80 lux). . . . , Water temperature; ■—■, L:D; Mi, micropter; Ma, macropter.

not only to demand cold treatment but also a succeeding period of illumination other than short or shortening.

Summarizing the information in Figs 1 and 2, a long, stationary illumination is usually not enough to avert diapause in *G. odontogaster* if it is a light-dark cycle of 18:6 or 19:5 or continuous illumination, or something in between (the curious 1,000 lux:80 lux rhythm). In some extreme cases of stationary illumination, however, the diapause reaction is ambivalent, guiding some larvae to immediately reproducing imagoes and others to diapause. Furthermore, a short day, even if lengthening, causes diapause. Therefore, to effectively avert diapause, a long, lengthening day rhythm is necessary. Remarkably, even under stationary light conditions the correlation between wing length and the reproductive stage is closely maintained, with a preponderance of long wings and diapause.

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Nitrosocarbyl as a Potent Mutagen of Environmental Significance

THE widespread accumulation of environmental pollutants has led to concern over their long-range effects on living organisms. Although many herbicides, pesticides, and food additives have no detrimental effect on experimental animals, the possible chemical interaction of these compounds with each other or with natural elements of the environment to produce biologically active compounds has rarely been considered. We have found that the combination of a food additive, sodium nitrite, with a commonly used pesticide, carbaryl, results in the formation in acid solution of a new mutagen of considerable potency, nitrosocarbyl (NC). A study of the reactions leading to formation of mutagens and carcinogens from several types of pesticides and herbicides is reported elsewhere¹. We present here an examination of the mutagenicity of NC in two bacterial systems, *Escherichia coli* and *Haemophilus influenzae*.

The preparation of a compound assumed to be nitrosocarbyl has been reported previously in a paper concerned with analysis of trace residues of pesticides². Formation of the nitroso derivative renders such compounds detectable at extremely low concentrations by polarimetric analysis. NC was prepared in quantity from carbaryl according to the following method. Twenty grams of N-methyl-1-naphthylcarbamate (carbaryl; Union Carbide Corporation, New York) was dissolved in 25 ml dimethylsulphoxide, and 10 g of ice was added, together with 20 ml acetic acid and 12 ml hydrochloric acid. Into the thick suspension was stirred 20 g sodium nitrite, the reaction mixture being cooled in an ice bath. The mixture was kept in a freezer for 2 d with occasional stirring, and an orange yellow colour slowly developed. After removal from the freezer, 200 ml ether was added, forming a deep orange upper layer, which was separated from the clear aqueous layer. The ether solution was dried with anhydrous magnesium sulphate, filtered, and evaporated to dryness in a stream of nitrogen at room temperature. The residual solid was dissolved in 30–40 ml ether, which was again evaporated in nitrogen. A first crop of cream yellow crystals was filtered off; these melted at 66–68°C, and the yield was 7.9 g. A second crop of crystals obtained by evaporating the mother liquid almost to dryness weighed 8.7 g and melted at 63–65°C. The total yield was 72%. The molar absorptivity of the first crop of

crystals was 144 at 402 nm in ethanol, and the absorption spectrum was almost identical with that of N-nitroso-N-methylurethane. The analysis calculated for $C_{12}H_{10}N_2O_3$ was C, 62.60; H, 4.37; N, 12.17; the analysis found was C, 62.60; H, 4.56; N, 11.99. The mass spectrum (70 eV) showed a parent ion at m/e 230 and fragments at m/e 143 (naphthoxyl) and m/e 127 (naphthyl). The compound, N-nitroso-N-methyl-1-naphthylcarbamate (nitrosocarbyl, NC), was fairly stable in the dry state and after standing in light and air at room temperature showed a drop in absorptivity of less than 10% after 3 days and 20% after 2 weeks.

To characterise its biological activity, the mutagenicity of NC was compared with that of nitrosomethylurethane (NMUr) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), the latter being a widely used mutagen in experimental systems. NMUr, a known mutagen and carcinogen³, is a structural analogue of NC. Decomposition of mutagenic nitroso compounds, thought to be an important factor in their biological activity⁴, was found for our compounds to be less than 5% in 1 h in treatment buffer (Tris-maleate, pH 6), as followed by ultraviolet absorbance. Since mutation/survival ratios tended to show maxima, we found it useful to compare our compounds on the basis of the relative concentrations required to give similar mutation curves. Survival was measured in each case, as seen in Fig. 1. In each experiment, a standard concentration of MNNG was tested as a positive control, against which the other compounds could be compared. An aliquot of cells was also treated with solvent alone for measurement of the spontaneous mutation rate, which can be used to calculate the induced level of mutation.

In general, the mutation rate increased as a function of

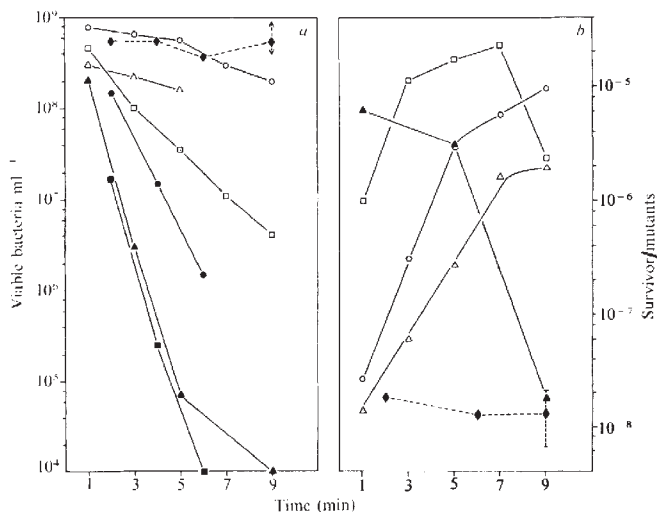


Fig. 1 Kinetics of survival (a) and mutation (b) of *H. influenzae* after treatment with NC, MNNG, and NMUr. *H. influenzae* (wild type strain Rd or a streptomycin-resistant derivative of Rd) was grown to log phase, pelleted and resuspended in 0.05 M Tris-maleate buffer (pH 6) (approximately 6×10^8 cells ml^{-1}), and exposed to the mutagen for a short time. Mutagens were dissolved and further diluted in ethanol, followed by a final 1:50 dilution into the bacteria. Treatment was stopped by ten-fold dilution (and further dilution and plating for survival determinations) and subsequent washing by centrifugation and resuspension of the pellet. Treated samples were then resuspended in growth medium and incubated at 37°C to allow expression of mutations. Cultures grown to a standard density were frozen in aliquots and later tested for mutation to novobiocin resistance by plating under novobiocin agar (2.5 μg ml^{-1}), as previously described⁵. a, Survival after treatment with 0.1 mM NC (■); 0.01 M NC (□); 1.0 mM NMUr (▲); 0.1 mM NMUr (△); 1.0 mM MNNG (●); 0.5 mM MNNG (○) or 0.1 mM carbaryl (◆). b, Mutation after treatment with 0.01 mM NC (□); 1.0 mM NMUr (▲); 0.1 mM NMUr (△); 0.5 mM MNNG (○); or 0.1 mM carbaryl (◆). (---) indicates area of untreated control. (---) indicates area of spontaneous mutation.

killing up to a loss of about 90% of the cells, after which the observed mutation frequency levelled off and then declined, presumably due to killing of mutants (Fig. 1a-b). The dependence of the shape of the curve on mutagen concentration is illustrated by the NMUr results at a ten-fold difference in concentration (Fig. 1b). The molar concentration of NC required to induce a mutation frequency of 10^{-5} is less than one-fiftieth the concentration of MNNG required, and less than one-tenth the concentration of NMUr required to induce a similar level of mutation. The precursor, carbaryl, was not mutagenic (Fig. 1b).

All three of the compounds tested are N-methyl-N-nitroso compounds. The difference in their potency may be due to differential formation of reactive intermediates within the cell or to differential uptake of the compounds, governed by the structure of the rest of the molecule. The presence of the naphthyl group on NC, which increases its lipid solubility, appears to be a factor in its biological activity.

E. coli strain H/r 30 R requiring arginine was used for measurement of mutation to prototrophy, according to procedures previously described⁶. *E. coli* was found to be less sensitive than *H. influenzae* to both MNNG and NC. NC at 0.1 mM and MNNG at 1.0 mM killed approximately 50% of the *E. coli* cells after a treatment time of 10 min. The induced mutation frequencies (reversion to prototrophy) for these treatments were 3×10^{-4} and 5×10^{-4} per survivor, respectively. Both killing and mutation were less sensitive functions of mutagen concentration and treatment time in *E. coli* than in *H. influenzae*. These results are in agreement with previous studies of MNNG in *E. coli*⁷.

Our two test systems were chosen arbitrarily with respect to both organism and genetic loci at which mutations were assayed. Since NC was more active than MNNG in both systems, we conclude that NC is a potent mutagen, which may be of interest to experimentalists studying mutation phenomena. NC is also a possible hazard to man, since its precursors carbaryl and nitrite are both common environmental constituents which are consumed (carbaryl as a crop residue⁸ and nitrite as a food additive), and the stomach provides the acidic conditions for its formation^{1,9}. This interaction is likely to be biologically significant because N-nitroso compounds are known to act systemically and to be most effective as carcinogens when administered in small doses over long periods of time. No animal species have yet been found to be resistant to the biological effects of N-nitroso compounds³, and on the basis of the known carcinogenicity and mutagenicity of its close structural analogue NMUr³, nitrosocarbaryl is likely to be a carcinogen as well as a mutagen.

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Physiological Variation in Circulating B Cell:T Cell Ratio in Man

T LYMPHOCYTES in human peripheral blood may be identified by their ability to form rosettes *in vitro* with untreated sheep erythrocytes¹⁻⁷. This test has been widely applied to detect disturbances in the B:T cell ratio in various disease states^{3,5,7-11}, there is, however, no published information on the stability or otherwise of the blood B:T cell ratio in health. Such studies are required because it is known that fluctuations occur readily in the numbers and proportions of the different classes of circulating leukocytes¹². Diurnal variation principally affects the granulocytes but exercise, emotion and the administration of adrenaline are all known to provoke a rapid and substantial lymphocytosis through mobilisation of cells normally sequestered in undetermined sites¹³⁻¹⁵. Here we report investigations on the effect of exercise.

Peripheral blood was obtained from six healthy young laboratory workers. From each sample an aliquot (0.5 ml) was mixed with a chelating agent (sequestrene) for determination of the total white cell count in an electronic counter and for making air-dried smears which were stained by May-Grunwald-Giemsa. The proportion of lymphocytes in the sample was determined by counting at least 500 cells from these smears. The remainder of each sample was defibrinated and lymphocytes were separated from it by centrifugation over a Ficoll/Triosil layer¹⁶.

The sheep-cell rosette technique used in this study has been described in detail elsewhere¹⁷. Erythrocytes from a selected animal were stored in Alsever's solution at 4° C for up to 2 weeks before use. Triplicate samples of human lymphocytes and sheep red cells were mixed in the presence of 10% foetal calf serum, sedimented at 50 g for 5 min and allowed to stand overnight before reading the percentage of rosettes in a total of at least 200 lymphocytes from each tube.

Starting at about 0930 h, each subject undertook 10 min of vigorous exercise (running upstairs). Blood was drawn immediately before beginning the exercise, 5 min after the end of it and 45 min, 2 h and 4 h from the start of the experiment.

In every case there was a marked lymphocytosis after exercise, most of the increase comprising non-rosette-forming cells (presumptive B cells) (Table 1a, Fig. 1). By 45 min the total lymphocyte count and the percentage of rosette-forming cells had returned to the pre-exercise level. In the later samples there was a polymorph leukocytosis of extremely variable degree.

The finding that the majority of rapidly mobilised lymphocytes are presumptive B cells is in keeping with other evidence that the influx of cells does not occur through the thoracic duct^{15,18} (the lymphocyte content of which comprises mainly T cells^{19,20}).

Control experiments, in which blood was drawn at the same time intervals but without the period of strenuous exercise, demonstrated that there is no consistent pattern of diurnal variation in the blood B:T cell ratio (Table 1b). Comparing the different samples from each subject, however, some fluctuation in the proportion of rosette-forming cells was observed. The largest range recorded was 11% (59.3 to 70.3%) and the smallest 5.4% (64.0 to 69.4%). We have previously noted a similar variation in the percentage of rosette-forming cells in blood samples collected on dif-

Table 1 Numbers of B and T cells in circulation

Time (min)	Total WBC mm ⁻³	Total Lymphocytes mm ⁻³	% RFC (range)	Total RFC mm ⁻³	Total non-RFC mm ⁻³
a					
0	4,088 (1,035)	1,632 (262)	61.8 (53.8 to 63.4)	1,012 (190)	620 (106)
15	5,547 (1,390)	2,757 (690)	46.4 (41.8 to 50.9)	1,273 (310)	1,484 (432)
45	4,416 (1,718)	1,693 (312)	60.2 (57.7 to 64.6)	1,017 (200)	675 (140)
120	5,201 (1,824)	1,889 (386)	56.3 (37.2 to 64.6)	1,050 (260)	840 (323)
240	5,573 (150)	1,754 (70)	60.6 (52.5 to 65.9)	1,062 (98)	692 (97)
b					
0	4,024 (654)	1,623 (245)	62.0 (55.4 to 67.6)	1,003 (170)	622 (140)
15	4,032 (626)	1,628 (318)	62.2 (58.1 to 66.9)	1,004 (139)	624 (182)
45	4,229 (432)	1,716 (262)	62.9 (59.3 to 69.4)	1,074 (131)	643 (149)
120	5,056 (1,114)	1,689 (119)	61.8 (53.5 to 70.3)	1,048 (148)	651 (149)
240	5,216 (905)	1,857 (169)	61.4 (57.3 to 66.1)	1,131 (31)	724 (143)

a, Response to exercise; b, Control experiments (no exercise).

Figures are mean values for a, Six subjects; b, Four subjects. Standard deviation in parentheses except for % RFC where range is quoted. WBC=white blood cells. RFC=rosette-forming cells.

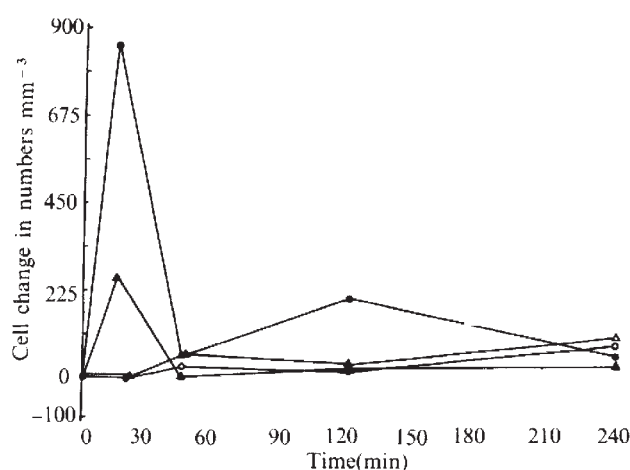


Fig. 1 Changes in total number of circulating RFC (T cells) (▲, △) and Non-RFC (B cells) (●, ○) following vigorous exercise (six experiments; ●, ▲) and in four controls (four experiments; ○, △). Pre-exercise level is taken as zero. Figures are calculated from electronic total white cell count, differential count of at least 500 stained leukocytes and mean of triplicate estimates for percentage of RFC.

ferent days from the same donors¹⁷. As the mean coefficient of variation for triplicate tubes in the rosette test was less than 5%, these findings seem to reflect a true and unpredictable fluctuation in the blood B:T cell ratio of healthy adults in conditions of normal daily activity.

In view of the demonstrated effect of strenuous exercise it seemed possible that the fluctuations observed in the control

experiments were also related to physical activity. Samples were therefore obtained from five of the subjects in near-basal conditions (before rising after a night's sleep). These samples were handled as described above and the RFC:non-RFC ratio compared with the values obtained in random daytime samples obtained over a period of several weeks from the same donors (Table 2).

In two cases (subjects B and D) the percentage of presumptive T cells in the early morning samples was greater than in any of their random specimens but in the other three subjects the figures obtained under near-basal conditions lay close to the mean values recorded during normal daily activity. When repeated early morning samples were examined from subject C, their variation in percentage of RFC was comparable to the variation observed in random daytime samples.

It therefore seems that the physical exertion undertaken in normal non-manual working conditions contributes little to the physiological fluctuations in the peripheral blood B:T cell ratio in health. The fluctuation does not follow any predictable time course and, apart from strenuous exercise, its causes have yet to be defined.

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Table 2 Percentage of rosette-forming cells among blood lymphocytes of five healthy donors under 'basal' conditions and in random daytime samples

Subject	Random samples*		No. of samples	'Basal' (early morning) samples		No. of samples
	Mean	(Range)		Mean (s.d.) † or (range)		
A	66.2	(61.8 to 69.4)	7	63.1 (2.3)		1
B	61.1	(53.5 to 67.7)	7	70.9 (3.3)		1
C	62.0	(51.9 to 70.3)	7	62.0 (55.7 to 72.5)		5
D	57.0	(53.8 to 61.0)	6	69.1 (2.2)		1
E	62.8	(54.4 to 68.4)	6	59.6 (1.6)		1

* Blood drawn at different times of day during normal activity, not involving strenuous exercise.

† Standard deviation of triplicate values for single blood sample.

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Promotion of Reddening in Unripe Strawberry Fruits by Fungal Extracts

ETHYLENE promotes ripening in many fruits^{1,2}, and auxins cause premature colouring in pears³, apples^{4,5} and other fruits⁶. Some pome fruits redden prematurely around damaged areas.

No reports have been found of promoted ripening or reddening of strawberry fruits by ethylene, auxins or damage, and attempts elsewhere^{7,8} and at Long Ashton, to advance ripening in strawberry by ethylene and by 2-chloroethylphosphonic acid (CEPA or Ethrel), which decomposes to yield ethylene in plant tissues, have not been successful, although tests have been done under several conditions, both *in vitro* and on fruit retained on the plant.

This investigation arises from the finding of several immature strawberry fruits, of the cultivar Rabunda, on which areas surrounding pathogenic infections were prematurely reddened. Lesions were pale-brown in colour, dry and firm, varying in size from about 1 to 3 cm² in area. The reddened areas surrounding the lesions formed a band up to about 1 cm in width. The remaining parts of the fruit were green and unripe at the time, but ripened later. A fungus was isolated from an infected fruit and identified as *Fusarium sambucinum* Fuckel, of importance on hops and stored potato but also recorded on strawberry in Europe⁹.

Cultures of the fungus were incubated either on potato dextrose agar for 14 d, or in Czapek Dox liquid media for 15 d at 25° C. Six agar cultures in 9 cm Petri dishes were macerated in 100 ml methanol and the liquid phase separated. The methanol was evaporated off at 40° C under nitrogen. The water fraction was made up to 100 ml with distilled water and its pH adjusted to 2.5 with 1% HCl. This water extract was partitioned three times against 50 ml of ethyl acetate and the ethyl acetate phases dried with a little anhydrous sodium sulphate. The ethyl acetate was evaporated off at 40° C and the residue preserved for bioassay. Liquid cultures were extracted in a similar way.

The residues were assayed for their ability to colour unripe strawberry fruits *in situ* on plants in the glasshouse. Suitable fruits were firm but not hard, pale-green in colour, and 5 to 7 d before the stage at which they would normally colour.

Individual achenes were removed from the fruits with a sterilised scalpel, and a small cavity opened up beneath the scar. The residues to be assayed were taken up in 20% ethanol in water, and 8 µl aliquots placed into freshly-opened cavities. Treatments and controls (the latter included application of 20% ethanol and extracts of uninoculated agar and untreated cavities) were replicated from twice to ten times in different tests.

Active extracts applied in the manner described induced a ring of precocious reddening in tissue round the cavity 1 to 4 d after application, the response time depending partly on the time of year. The rings of reddened tissue were 2 mm wide and up to 1 cm in diameter, the diameter being less with greater dilution of the extracts. The cavity was separated from the ring by a zone of decoloured tissue, which did not ripen. Normal ripening of the fruit occurred outside the ring, which was no longer distinguishable, 3 to 5 d later. Control cavities exhibited

neither red rings nor decoloured zones: the lip and lining inside the cavity turned brown but did not rot.

The dependence of ring diameter on extract concentration and the presence of the inner zone of decoloured tissue, indicate that the extracts may have been toxic in high concentrations near the point of application but became diluted by diffusion outwards to the region where they induced pigment formation in the host tissue.

Bioassay on *Avena mesocotyls* indicated that the extracts contained growth-regulating substances.

In work of colleagues in this laboratory, similar rings of reddened tissue were induced by the application of several auxins. Therefore, it appears likely that the fungal extract contained auxins or possibly other unidentified substances which can initiate or promote reddening or ripening in strawberry. The failure of attempts to promote ripening by ethylene or ethylene generators, however, raises the question whether ethylene was mediating in the stimulation of reddening either by auxins or the fungal extract, even though auxins are known to stimulate ethylene evolution in several plant tissues^{10,11} and ethylene to promote ripening in many fruits.

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Negative Cooperativity of Phosphofructokinase as a Possible Regulator of Ripening in Banana Fruit

THE banana belongs to a group of fruits which undergo a rapid increase in respiration with ripening¹. Several theories have been advanced to account for this 'respiratory climacteric': the most often mentioned hypotheses are (1) the uncoupling of oxidative phosphorylation, (2) a change in acceptor control by ADP, (3) a change in membrane permeability, and (4) the synthesis or activation of a rate-limiting enzyme. The uncoupler theory and the ADP to ATP ratio change have, however, been disproved, and no clear evidence has been presented in support of the other theories.

In lacatan bananas a twenty-fold increase in the fructose-1,6-diphosphate level has been measured and it has been suggested that this could indicate the activation of phosphofructokinase². Activation of this same enzyme by an increasing concentration of inorganic phosphate has been suggested as a possible reason for increased respiratory activity in ripening

tomato fruit⁴. Our study reports the regulatory control properties of phosphofructokinase during ripening of banana fruit.

The respiration of individual banana fruits *Musa cavendishii* Lambert var. Valery was followed with an infrared gas analyser in a continuous flow system. Fruit were removed from the respirometer (1) in the preclimacteric stage, (2), on respiratory rise, (3) at climacteric peak, and (4) at the two-day post-climacteric stage; they were then peeled and the pulp was grated into liquid nitrogen. Frozen tissue was lyophilised and stored at -12°C . The dry pulp tissue was ground to a fine powder in a dry mortar. The powder for each of the four stages was extracted twice with extraction medium for the determination of endogenous metabolite levels. The extraction medium consisted of 50 mM tricine buffer, pH 8.5, 5 mM diethyldithiocarbamate, 5 mM dithiothreitol, 50 mM magnesium acetate and 0.2% Triton X-100. Stage 1 and stage 3 powders were extracted with the same medium and purified five-fold for phosphofructokinase assays.

Quantitative analysis of glycolytic intermediates showed the greatest relative change (twenty-fold) in the level of fructose-1,6-diphosphate between the preclimacteric stage to the climacteric peak; fructose-6-phosphate and glucose-6-phosphate, however, showed less than a one-fold increase (Table 1). This is consistent with phosphofructokinase activation hypothesis.

Table 1 Changes in levels of some glycolytic intermediates between preclimacteric and climacteric peak stage

Substrate	Preclimacteric (nmol g ⁻¹)	Climacteric peak (nmol g ⁻¹)	Change (%)
Glucose-6-phosphate	443 ± 23	792 ± 40	+79
Fructose-6-phosphate	444 ± 17	565 ± 30	+27
Fructose-1,6-diphosphate	7 ± 2	147 ± 11	+2,100
Triosephosphate	61 ± 16	227 ± 10	+372
Phosphoenolpyruvate	163 ± 9	533 ± 17	+327
Pyruvate	125 ± 8	366 ± 5	+293

Mean and standard error of duplicate samples are given.

The response of a partially purified phosphofructokinase from the preclimacteric (stage 1) and climacteric peak (stage 3) powder to increasing fructose-6-phosphate concentration, is shown in Fig. 1. The preclimacteric enzyme yielded a substrate saturation curve which is neither hyperbolic nor sigmoidal, whereas the climacteric peak enzyme displayed a more nearly hyperbolic pattern. The enzyme from both sources required very high substrate concentration to reach saturation. Figure

1 also shows that citrate, which has been shown to inhibit phosphofructokinase from carrot⁵ and pea⁶, did not inhibit at 5 mM concentration. A small inhibitory effect at very low fructose-6-phosphate concentration is shown in the insert to Fig. 1, but this is not large enough to regulate respiration. Other compounds reported as allosteric effectors of phosphofructokinase, such as inorganic phosphate, ammonium ion, ATP and ADP, showed no response or gave the same response with the enzyme from both sources. Plotting the data according to the Eadie equation⁷ yielded curves which were concave upward (Fig. 2). The slow rate of substrate saturation and

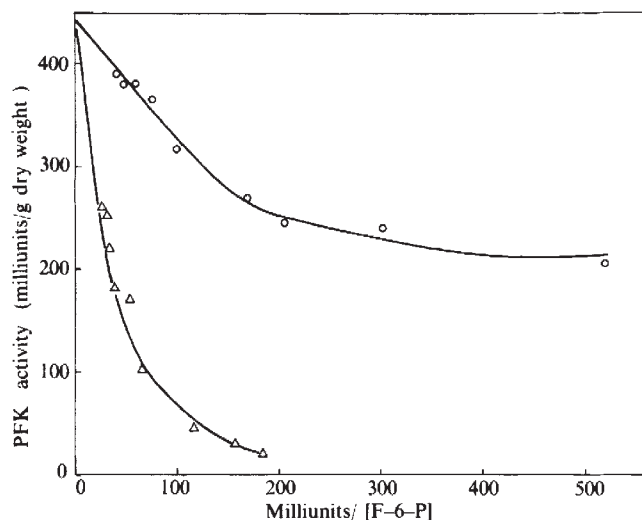


Fig. 2 A plot of the data of Fig. 1 according to the Eadie equation, $v = V_{\max} - K_m (v/S)$.

the shape of the Eadie plots are most consistent with the kinetic behaviour described as negative cooperativity⁸. According to this model the binding of one molecule of substrate makes the binding of the next molecule at another site on the same enzyme more difficult. This will maintain metabolism of carbohydrate through glycolysis at a low rate in spite of large changes in substrate. The pentose phosphate pathway, which provides NADPH and intermediates for RNA synthesis, can therefore proceed relatively independently of glycolysis.

The estimates of the $V_{\max}$, the substrate concentration giving maximal reaction velocity, based on the Eadie plots, indicated that both the preclimacteric and the climacteric peak enzyme had the same value: 412 milliunits per g dry weight of powder. The $(S)_{0.5}$ value, the substrate concentration giving half-maximal activity, however, was 5 mM for the preclimacteric and 1 mM for the postclimacteric phosphofructokinase. We suggest that there is neither synthesis of a new kind of enzyme nor of the same enzyme, but rather that the activity of preclimacteric phosphofructokinase is kept low by the negative cooperative effect. This control is partially desensitised in some unknown way at the initiation of the climacteric.

Extraction procedures have been known to desensitise regulatory enzymes. We feel confident that control of the climacteric peak enzyme has not been affected by the procedures used because the enzyme does show some degree of control which is reproducible from experiment to experiment. The purification procedure includes an ammonium sulphate fractionation and a 3 min, 59°C heat treatment which leaves the control properties of both enzymes unchanged. Further, *in vivo* product accumulation agrees with the change in control of the extracted enzymes.

To our knowledge this is the first case where negative cooperativity has been shown in phosphofructokinase. Our results indicate that the behaviour of the banana phospho-

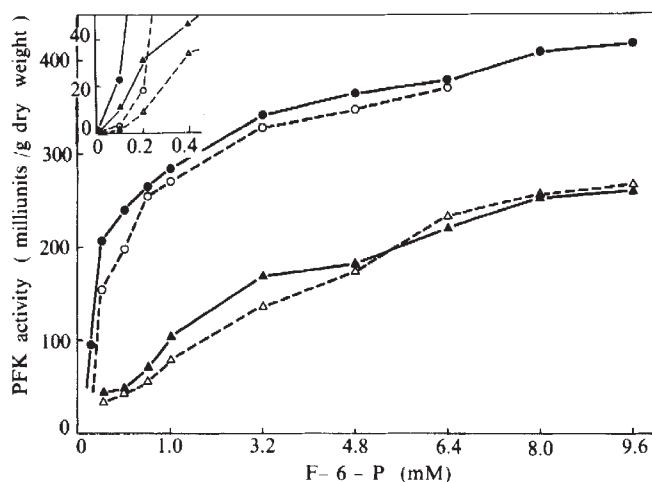


Fig. 1 Activity of phosphofructokinase (PFK) from preclimacteric and climacteric peak banana fruit as a function of fructose-6-phosphate (F-6-P) concentration. ●—●, Climacteric peak; ○—○, with 5 mM citrate; ▲—▲, zero time; △—△, with 5 mM citrate.

fructokinase is consistent with the activation hypothesis and that it can account for the observed five-fold increase in the respiration during the climacteric.

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Glycoprotein in the Capsid of Plant Viruses as a Possible Determinant of Seed Transmissibility

It is well established that only certain plant viruses are transmitted by the seed, but little is known about how a virus invades the embryo and is subsequently transmitted to progeny^{1,2}. Since viral coat proteins are determinants of host³ and vector⁴ specificity, we reasoned that they could also determine transmissibility. Accordingly, coat proteins of two seed-transmitted viruses, barley stripe mosaic virus (BSMV) and cowpea mosaic virus (CPMV), and three non-seed-transmitted viruses, tobacco mosaic virus (TMV), bromegrass mosaic virus (BMV) and bean pod mottle virus (BPMV)^{1,2}, were analysed.

BSMV, BMV and TMV were purified by centrifugation through multiple sucrose density gradient columns similar to those used in methods described previously⁵. CPMV and BPMV were purified by chloroform-butanol extraction, three cycles of differential centrifugation⁶ and sucrose density gradient electrophoresis. Purified virus was not further separated into its electrophoretic forms⁷. Viral coat protein was isolated from highly purified BSMV and BMV by 1 M CaCl₂ (ref. 5), from CPMV and BPMV by the guanidinium LiCl method of Wu and Bruening⁸ and from TMV by the acetic acid method of Fraenkel-Conrat⁹. The coat protein from each virus was analysed for carbohydrate as described in Table 1.

Table 1 shows the carbohydrate composition of the two seed-transmitted viruses, BSMV and CPMV. Both contained glucose, mannose, glucosamine and galactosamine, while xylose was present only in BSMV and galactose only in CPMV. Sialic acid, a common component of animal glycoproteins, was not detected. The coat proteins of the three non-seed-transmitted viruses contained no detectable carbohydrate.

Conditions for optimal carbohydrate release vary with each glycoprotein and depend on the stability of the glycoside linkages as well as the resistance to acid degradation of the released sugar residues. The hydrolysis conditions noted in Table 1 have been used successfully for various glycoproteins¹⁰. Even so, the values noted are minimum values since they represent the difference between the quantity of sugar released and quantity degraded during hydrolysis. Hydrolysis condi-

tions normally used for amino acid analysis (6 N HCl, 100° C, 24 h) have been shown to degrade amino sugars extensively¹⁰. Our amino acid analysis of BSMV showed only a trace of amino sugars after 12 h of hydrolysis, and absence after 24 and 72 h of hydrolysis. This probably explains why previous amino acid analyses of BSMV⁵ and CPMV⁷ detected no amino sugars.

Table 1 Carbohydrate composition of BSMV and CPMV

Carbohydrate *	BSMV		CPMV	
	g per 100 g coat protein	Mol per virus particle	g per 100 g coat protein	Mol per virus particle
Glucose	1.36	1,830	0.51	136
Mannose	0.25	336	0.14	37
Xylose	0.38	614	—	—
Galactose	—	—	0.19	51
Glucosamine	0.66	891	0.70	187
Galactosamine	1.06	1,430	0.36	297
Sialic acid	0.0	0.0	0.0	0
Total	3.71	5,101	1.90	708

* Neutral sugars analysed by gas liquid chromatography of alditol acetate derivatives¹⁹ after hydrolysis in 0.5 N H₂SO₄ containing Ag-50 × 8 (H⁺), 100° C, 24 h; amino sugars were analysed by amino acid analyser and by gas-liquid chromatography of aminitol acetate derivatives¹⁹ after hydrolysis in 4 N HCl, 100° C, 24 h; sialic acid was analysed using thiobarbituric acid²⁰ after hydrolysis in 0.1 N H₂SO₄, 80° C, 1 h. The alditol and aminitol acetates were chromatographed in a 2.6 mm 2 m glass column containing 3% ECNSS-M on 100/120 Gas-chrom Q isothermally at 180° C for the alditol acetates and at 200° C for the aminitol acetates. The quantity of each sugar was determined by comparing the area under its peak with that of the internal myo-inositol standard. The identity of each peak was confirmed using gas liquid chromatography-mass spectroscopy by comparing its retention time and computer print-out of the mass spectrum with known standards.

BSMV is a 128 × 20 nm rod¹¹ composed of 1,100 identical protein subunits¹² which account for 96% (w/w) of the virus particle¹³. The 3.71% carbohydrate in the coat protein indicates that this virus may contain up to 5,000 mol of carbohydrate per virion. CPMV is a 28 nm diameter icosahedral particle¹⁴ composed of 180 protein subunits which account for 71.5% (w/w) of the virus particle¹⁵. Correspondingly, the 1.90% carbohydrate in the coat protein suggests that this virion contains up to 700 mol of carbohydrate. The presence of these rather large amounts of carbohydrate could convey to these viruses a unique capacity for interaction with their hosts.

It is significant that the two viruses with a glycoprotein coat are seed-transmitted while the three viruses devoid of carbohydrate residues are not^{1,2}. Furthermore, CPMV and BPMV are very closely related in that they: (a) cross-react serologically¹⁶; (b) have similar host ranges; (c) have a similar complement of fast and slow electrophoretic forms and subunit proteins^{10,17}, and (d) have similar amino acid compositions in their coat proteins^{10,17}. Thus when the properties of these two closely related viruses are compared it seems particularly relevant that seed-transmitted CPMV has a glycoprotein coat, while non-seed-transmitted BPMV has a coat protein devoid of carbohydrate.

Because viral invasion of gametophytic tissues is a prerequisite for seed transmission^{1,2,18}, seed-borne viruses seem to possess a unique property which enables them to invade reproductive tissues. We suggest that this unique property of seed-borne viruses is related to the glycoprotein portion of the coat protein. More specifically, the covalently linked carbohydrate residues on the viral coat may function as recognition sites to attach the virus to gametophytic cell surfaces and thereby facilitate viral invasion of reproductive tissues and subsequent seed transmission.

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Glutathione Peroxidase Activity as a Function of Dietary Selenomethionine

SINCE 1957, selenium has been recognized as an essential micronutrient for animals, but its exact biological function has remained uncertain. The metabolic role of selenium in animals seems to be linked with vitamin E and sulphur amino acids¹⁻⁴. Selenium has a sparing effect on vitamin E, and delays the onset of deficiency syndromes. Likewise, vitamin E and sulphur amino acids partially protect against or delay the onset of several forms of selenium deficiency syndromes. Rotruck *et al.*⁵ proposed that selenium functions as an integral part of glutathione (GSH) peroxidase, an enzyme that reduces toxic lipid peroxides to hydroxy acids⁶⁻⁸. They found that a large proportion of the ⁷⁵Se from rat erythrocytes labelled *in vivo* remained with the enzyme during extensive purification, and more recently, their studies with highly purified GSH peroxidase have indicated that it contains approximately 4 mol of Se per mol of enzyme⁹. Similar findings were reported by Flohé *et al.* for bovine erythrocyte GSH peroxidase¹⁰. To learn more about the nutritional relationship between selenium and GSH peroxidase, we performed studies that showed GSH peroxidase activity is proportional to dietary selenium, which provided additional evidence for the role of selenium in the function of GSH peroxidase.

Weanling male Sprague-Dawley rats were fed a selenium-deficient diet that contained sucrose, 43%; *Torula* yeast, 36%; stripped corn oil, 10%; stripped lard, 5%; salt mix, 5%; and vitamin mix, 1%. This diet was supplemented with 0, 0.05, 0.2, or 2.0 p.p.m. selenium as selenomethionine. After 5 weeks, the animals were exsanguinated by heart puncture.

The heparinised blood was centrifuged at 3,000 r.p.m. for 25 min. The packed cells were washed with isotonic buffer and lysed with hypotonic phosphate buffer. The tissues were stored frozen pending analysis. Tissues from individual animals were prepared as approximately 6% homogenates in isotonic potassium phosphate buffer, pH 7.0. After centrifugation for 10 min at 750g, the supernatant was re-centrifuged for 60 min at 100,000g to obtain the soluble fraction. GSH peroxidase activity in the soluble fraction was assayed at pH 7.6 by a modification of the method of Paglia and Valentine¹¹, with cumene hydroperoxide as substrate. The activity was measured spectrophotometrically as the rate of NADPH oxidation through the coupled reaction with excess GSH reductase. Previous work showed that the activities of GSH reductase and glucose-6-phosphate dehydrogenase, as well as GSH peroxidase, increased significantly in response to oxidative stress^{12,13}; therefore, these enzymes were measured as controls^{14,15} to show the specificity of selenium for GSH peroxidase. Protein concentration in the soluble fractions was also determined¹⁶.

In all tissues, dietary supplementation with 0.2 p.p.m. and 2 p.p.m. selenium significantly ($P < 0.01$) increased the activity of GSH peroxidase over the activity in tissues from non-supplemented controls. Additionally, supplementation with 0.05 p.p.m. selenium significantly ($P < 0.01$) increased the enzyme activity above that of non-supplemented animals in all tissues except liver. Linear regression analysis showed that the increased GSH peroxidase activity was a function of the logarithm of the dietary selenium concentration (Fig. 1). All correlation coefficients were above 0.7 and all

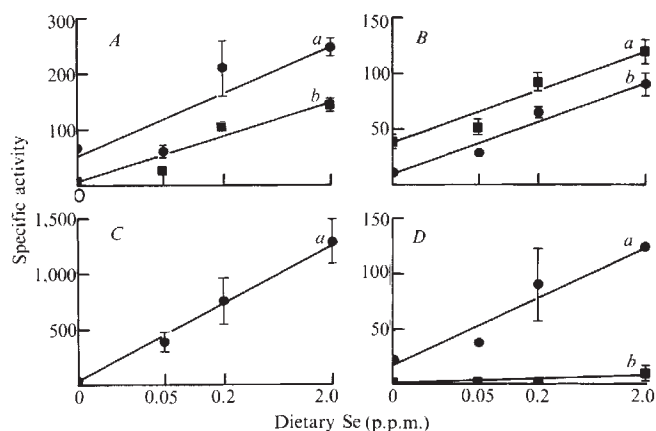


Fig. 1 Relationship of GSH peroxidase activity to dietary selenium. Specific activity is expressed as nmol NADPH oxidised per min per mg of protein for all tissues. Plasma-specific activity is expressed as nmol NADPH oxidised per min per ml. Each data point is the mean for nine animals in the 0 p.p.m. selenium group and for five animals in each selenium supplemented group. Bars represent $\pm$ standard deviation, points that lack bars have standard deviations smaller than the graphical representation of the data point. A: a, Liver; b, heart. B: a, RBC; b, lung. C: a, Plasma. D: a, Kidney; b, muscle.

$P < 0.001$. The relative increase of GSH peroxidase activity with dietary selenium supplementation during the experimental period was greatest in plasma, with other tissues following in the order liver > heart > kidney > erythrocytes $\approx$ lung > muscle. The close relationship between dietary selenium and tissue GSH peroxidase activities, particularly in the plasma, could be used as the basis for a rapid monitoring method for the selenium status of an animal. In all tissues studied, the activities of GSH reductase and glucose-6-phosphate dehydrogenase were unaffected by dietary selenium.

A similar specific effect of dietary selenium on GSH peroxidase activity was observed in chicks¹⁷ (S. T. Omaye, unpublished results). The mechanism of enhancement of GSH peroxidase activity has not been elucidated, although it is suspected to occur through increased synthesis of GSH peroxidase. In support of this mechanism for the enhancement of activity, our experiments indicate that selenium is not a dialysable or exchangeable cofactor; it is reported to be an integral part^{9,10} of the enzyme.

Use of similar nutritional studies to yield evidence of the involvement of a trace element with a specific enzyme is well documented¹⁸; for example, xanthine oxidase in rats fed a highly purified diet was shown to be proportional to dietary supplementation with a liver residue factor that later was identified as molybdenum¹⁹.

The critical dependency of GSH peroxidase activity on dietary selenium may explain the long observed and puzzling interactions among dietary selenium, vitamin E and sulphur amino acids. GSH peroxidase, especially in the absence of dietary vitamin E, plays a major role in detoxifying lipid peroxides^{6-8,12} by reducing them to non-toxic hydroxy fatty acids, which prevents their decomposition into free radicals that can reinitiate peroxidation. The antioxygenic action of sulphur amino acids^{3,20} can be explained in part by their involvement in the production of GSH, which is linked through GSH reductase to the NADPH generating system of the cell. Hence, dietary selenium, sulphur amino acids, and vitamin E act synergistically to protect tissues from oxidative damage.

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Zooplankton Feeding on Differentially Labelled Algae and Bacteria

HERBIVOROUS zooplankton can graze on planktonic algae, bacteria and detrital particles¹. Selective feeding of planktonic crustaceans on algae has been described and attributed to passive size selection by filtration or raptorial feeding¹. The ingestion and utilisation of algae, bacteria and detritus by zooplankton has also been noted²⁻⁶ but as yet there have been few reports concerning the behaviour of zooplankton grazing on mixtures of algae and bacteria.

The potential importance for herbivorous zooplankton of food sources other than phytoplankton was demonstrated by Provasoli, Shiraishi and Lance⁷ who found that axenic cultures of the shore pool Copepod, *Tigriopus*, which did not remain viable for many generations if grown on a single strain of bacterial free algae, could be maintained indefinitely when their diet was supplemented with bacteria. In natural waters both the quantity and quality of zooplankton food sources are critical factors which are difficult to evaluate by present techniques⁸. Here we describe a method of differential radioactive isotopic labelling of algae and bacteria for studies of zooplankton grazing patterns and present some preliminary results using this method with zooplankton from Lake Kinneret (The Sea of Galilee), Israel.

During the summer, in the metalimnion of Lake Kinneret, the photosynthetic, green sulphur bacterium, *Chlorobium phaeobacteroides* can reach concentrations as high as 10^7 cells ml⁻¹. Large numbers of Cladocerans and early stages of Copepods are generally found in the upper metalimnic layer at this time⁹. Feeding experiments showed that the nauplii stages of the most abundant Copepod (*Mesocyclops leukartii*, Claus) developed normally when fed on *Chlorobium* isolated in pure culture on the enrichment medium described by Pfennig¹⁰. The rate of nauplii development on a *Chlorobium* diet was more rapid than on other bacterial and algal food sources (Table 1). It seemed reasonable, therefore, to investigate the possibility that, in this lake, as elsewhere¹¹⁻¹³, the large population of photosynthetic bacteria was being utilised as a food source by the zooplankton. Subsequent feeding experiments were made with *Ceriodaphnia reticulata* (Jurine) because this Cladoceran is one of the most common herbivorous zooplankters in Lake Kinneret. *Ceriodaphnia* and *Daphnia magna*, when taken from the lake, survived and reproduced normally for at least several weeks on diets of *Chlorobium*, *Chlorella vulgaris* or *Chlamydomonas reinhardtii* in filtered Kinneret water.

Table 1 Development time (d) in nauplii of *Mesocyclops leukartii* (Claus) on various food sources*

Food	Incubation temperature	
	15° C	27° C
<i>Chlorella vulgaris</i>	37 (±4)	12 (±2)
Rice bacteria †	36 (±7)	9 (±2)
Yeast		11 (±1)
<i>Chlorobium phaeobacteroides</i>	26 (±10)	8 (±1)

*In each experiment, which was repeated at least twice, ten nauplii were incubated in 15 ml filtered lake water. Food sources were added in excess (such as *Chlorobium* 2.5×10^7 cells ml⁻¹) as determined experimentally. The development times were averaged for all organisms in each experimental situation. Standard deviations in parentheses.

† Bacteria obtained from rice infusion.

Chlorobium phaeobacteroides is capable of assimilating organic substrates such as glucose, amino acids or acetic acid in the light¹⁴. Therefore, for our experiments, we labelled the bacteria by growing them with ³H-acetate (0.2 μC ml⁻¹) on Pfennig's medium¹⁰. Algal cultures (*Chlorella* or *Chlamy-*

Table 2 *Ceriodaphnia reticulata* feeding experiments with radioactively labelled algae and bacteria*

Experimental time (h)	No. of <i>Ceriodaphnia</i>	Food source (cells ml ⁻¹)		Assimilated cells per individual <i>Ceriodaphnia</i> †	
		Algae	Bacteria	Algae	Bacteria
4	50	61.4 × 10 ⁴	—	2.2 × 10 ³ (0.18)	—
4	50	—	2.0 × 10 ⁷	—	1.3 × 10 ⁴ (0.033)
4	50	61.4 × 10 ⁴	2.0 × 10 ⁷	0.2 × 10 ³ (0.02)	1.5 × 10 ⁴ (0.037)
4	50	267.2 × 10 ⁴	—	0.6 × 10 ³ (0.11)	—
4	50	—	23.3 × 10 ⁷	—	3.1 × 10 ⁴ (0.007)
4	50	267.2 × 10 ⁴	23.3 × 10 ⁷	0.2 × 10 ³ (0.037)	2.1 × 10 ⁴ (0.005)
18	20	104.4 × 10 ⁴	—	31.2 × 10 ³ (0.60)	—
18	20	—	52.5 × 10 ⁷	—	85.0 × 10 ⁴ (0.32)
18	20	104.4 × 10 ⁴	52.5 × 10 ⁷	4.2 × 10 ³ (0.08)	118.0 × 10 ⁴ (0.45)
40	20	40.0 × 10 ⁴ †	—	15.0 × 10 ⁴ (7.5)	—
40	20	—	0.6 × 10 ⁶	—	0.6 × 10 ⁴ (0.20)
40	20	40.0 × 10 ⁴ †	0.6 × 10 ⁶	0.4 × 10 ⁴ (0.2)	0.8 × 10 ⁴ (0.26)
43	50	2.0 × 10 ⁴	—	15.3 × 10 ³ (38.2)	—
43	50	—	35.0 × 10 ⁷	—	8.0 × 10 ⁴ (0.11)
43	50	2.0 × 10 ⁴	35.0 × 10 ⁷	0.3 × 10 ³ (0.75)	7.2 × 10 ⁴ (0.10)

* Algae and bacteria were labelled with ¹⁴C and ³H respectively as described in the text. Algal cells were counted in a haemocytometer and bacteria by specific absorbance of bacterial chlorophyll at 715 nm or by serial dilutions in agar shake tubes. Radioactivity determinations were made with a scintillation counter using Instagel fluor (Packard). Initial activities ranged from 2 to 350 c.p.m. per 10³ algal cells, and from 100 to 6,000 c.p.m. per 10³ bacteria.

† *Chlamydomonas* (*Chlorella*) were used in all other experiments.

‡ Percentage of food source utilised in parentheses.

domonas) were incubated with ¹⁴C bicarbonate (1 µC ml⁻¹) added to a mineral growth medium (Bristol).

The results of five experiments with *Ceriodaphnia* feeding on algae, or bacteria, or both, are shown in Table 2. In each experiment twenty to fifty *Ceriodaphnia* were incubated with slow rotary agitation in 100 ml of filtered lake water, to which were added algae, or bacteria, or both. For each experimental situation, the radioactivity in three replicates of five animals was determined. The uptake of algae or bacteria is given as cells per individual *Ceriodaphnia*. These values might also be expressed in terms of fresh weight biomass (for example, 650 bacterial cells=one algal cell), carbon biomass, or calorific value. None of these parameters, however, reflect the actual dietary value of the algae and bacteria for *Ceriodaphnia*. No significant radioactivity was retained in control samples with animals poisoned by 4% formaldehyde.

In all experiments, when mixed diets were given, the *Ceriodaphnia* assimilated much less algae than when fed on algae alone. In contrast, the utilisation of bacteria was relatively unchanged when algae were also included in the diet. These experiments indicate, therefore, a preferential assimilation of bacteria by *Ceriodaphnia*. In all cases, both algae and bacteria were in excess supply, as indicated by the very low values for percentage utilisation of each food source.

In *Ceriodaphnia* the ingestion of food is a rapid process, taking less than 5 min (ref. 15). Because all the present experiments lasted 4 h or more, it is probable that the results shown in Table 2 actually represent assimilated foodstuff. Discriminatory grazing in *Ceriodaphnia* seems unlikely^{1,15} and it would thus appear that the animals could more easily digest cells of bacteria than those of algae. One reason for this may be the relative ease with which the *Chlorobium* cells are lysed (Pfennig, personal communication) as contrasted to algae with cell walls of cellulose.

The above experiments give a preliminary indication of the preferential assimilation of bacteria by *Ceriodaphnia* and imply that the photosynthetic green sulphur bacteria of Lake Kinneret may indeed be a significant dietary source for zooplankton. More importantly, we illustrate that the simple technique of labelling algae with ¹⁴C, and bacteria (or other heterotrophs) with ³H can make possible more extensive and complex experiments to elucidate the patterns of zooplankton feeding in nature.

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Feeding Posture of Modern Stalked Crinoids

Most modern crinoids (Echinodermata) are comatulids, which lack the stalk characteristic of Palaeozoic crinoids. The specialisation and adaptation to different ecological niches made possible by loss of the stalk have resulted in several different ways of spreading the arms and pinnules in an array for suspension feeding. Until the 1960s, most observations were based on specimens in aquaria. These suggested that the comatulids sit on the substratum with the mouth orientated upwards, the arms stretched out in the form of a

broad funnel or collecting bowl. The crinoid was thought to wait passively for a rain of its food material from above¹. This feeding posture is used by some deep water crinoids^{2,3}. Direct observations of the feeding behaviour of a shallow water comatulid in the Red Sea during the 1960s demonstrated that the arms are withdrawn during the day, but at night are arrayed in a vertical plane to form a filtration fan. This fan is orientated perpendicular to a prevailing weak current, filtering some 40,000 l of water at a current speed of 2 cm s^{-1} during one period of activity⁴. These observations and a review of deep-sea photographs indicated that modern crinoids favour an environment with moderate currents and are to some degree current-seeking (rheophilic)⁵. Application of these ideas to the palaeoecology of Palaeozoic stalked crinoids suggested that most were rheophilic, using the stalk to raise the calyx above the substratum and allowing the arms to be outspread in a planar, circular filtration fan². The morphology of some ancient forms, however, was considered indicative of a rheophobic mode of life.

Direct observations of the shallow water crinoids of the West Indies using Scuba has expanded and refined knowledge of the various comatulid postures⁶. Some rheophilic comatulids which form a filtration fan are attached to elevated objects and favour habitats swept by moderate currents. In these species, as in the Red Sea comatulid, the arms are held such that the food groove faces downcurrent. Other West Indian comatulids attach the calyx within a cryptic niche but extend the arms in a fan normal to bidirectional wave surge at shallow depths. Rheophobic species may co-exist with the rheophiles, attaching within the reef infrastructure and extending the arms in several directions. The pinnules of each arm are arrayed in four directions, thus allowing effective filtration of slow, multidirectional water movements⁷. The formation of collecting bowls dependent on gravitational settling is apparently restricted to deep water species^{2,3}.

In August 1972, an extended series of dives in the submersible Nekton Gamma on the north coast of Jamaica at Discovery Bay, revealed an abundant fauna of living stalked crinoids and comatulids at depths of 200–300 m, the latter being the maximum operating depth of the submersible.

Extensive direct observation and photography by ourselves and others has demonstrated the formation of filtration fans by three species. The most prominent and largest of these is *Cenocrinus asterius* (L.), with nearly fifty arms (Fig. 1a, c). The stalk may approach 1 m in length. The arms were always seen to recurve aborally through almost 270° . In this posture the repeated branching of the arms and the pinnules form a virtually continuous parabolic filtration fan. The calyx with arms recurved in this manner was observed with the mouth orientated laterally (Fig. 1b) or upwards (Fig. 1e), appearing in the latter case like a wilted flower. The lateral tilting of the calyx (Fig. 1d) suggests current moving from left to right, but direct observations indicate that in fact the arms are recurved into the current, even in currents exceeding 0.5 knot. Thus the food grooves of the arms are directed downcurrent as in some rheophilic comatulids. The 'holding' power of the ligaments in the stalk is sufficient to keep the calyx erect even in currents, as suggested by histological studies⁶. In conditions of slack current, the calyx is horizontal, with the mouth upward. Comatulid crinoids were observed attached to the stalk of the stalked species, also forming a filtration fan (planar rather than parabolic) orientated like that of the 'host' stalked crinoid. Other comatulids attached to the substratum were also observed forming filtration fans in the same area.

Three other stalked crinoids were also present. *Endocrinus parrae* (Gervais), another large multi-armed species, arrayed the arms in a parabolic filtration fan like that of *C. asterius*. The small, five-armed *Democrinus* sp. was abundant at 240–300 m. It also forms the fan, but it is not as dense a screen as that of the former species. The calyx was observed to bend downcurrent, with the fan extended against the flow (Fig. 1g). In contrast to the three long-stalked crinoids, *Holopus rangii* d'Orbigny did not form a filtration fan. This species occurs commonly at depths of 270–300 m off Discovery Bay, cemented like barnacles to vertical rock outcrops. The ten short, massive arms were observed extended in a funnel-like arrangement which was rapidly infolded on approach of the submersible, giving the appearance of a clenched fist.

Anchorage of the two large species, *C. asterius* and *E. parrae*, is achieved solely by the cirri along the stalk. The stalk curves onto the substratum and four or five groups of cirri grasp the substratum with the terminal claws (Fig. 1f). The stalk terminates abruptly and seems not to give rise to an actual root. Some cirri along the vertical part of the stalk near the substratum seem to act as props (Fig. 1f).

Photographs obtained (by C. Neumann, personal communication) from the submersible Alvin in the Straits of Florida at 550 m demonstrate that stalked crinoids form filtration fans with the food grooves downcurrent in that location also. The genera present seem to be *Endocrinus* and *Democrinus*. Some comatulids present also form filtration fans. Sediment shadows behind sponges and sponge growth forms confirm current directions.

Several conclusions are suggested by the observations in Jamaica and the Straits of Florida. The stalked crinoids of the West Indies usually form filtration fans for suspension feeding in areas swept by moderate currents. The presence of these species in deep-sea photographs and dredge samples suggest the occurrence of moderate bottom currents. The orientation of the arms establishes current direction. The cirri are the means of anchorage in the larger species, serving also as props for the stalk. The stalk ligaments provide sufficient rigidity to hold the calyx erect even under moderate currents. It is thus highly probable that Breimer's inferences² as to the rheophilic habits of many Palaeozoic crinoids are correct. Other specialised Palaeozoic stalked crinoids may have used some of the same diverse patterns as those of modern unstalked forms.

We thank Dr L. S. Land (Department of Geology, University of Texas), who made time available in the Nekton Gamma

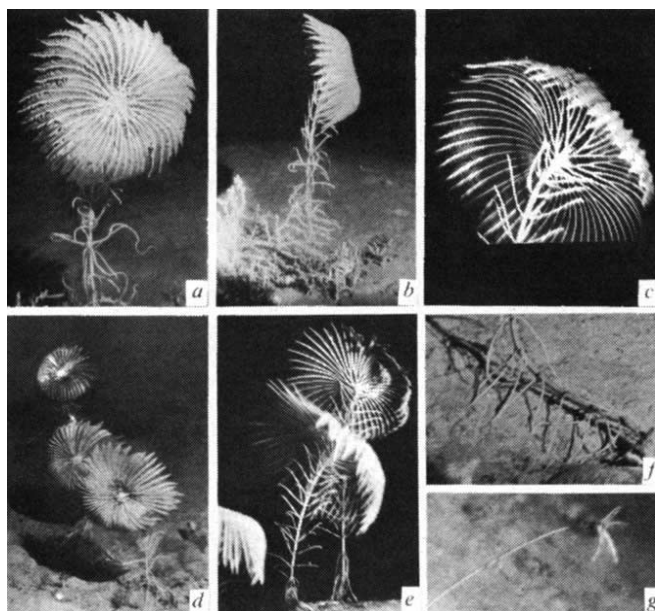


Fig. 1 *In situ* views of *Cenocrinus asterius* (L.) between 200 and 300 m depth, off Discovery Bay, Jamaica. Total vertical height of animals approximately 1 m (a–e); f, basal part of stalk with cirri, supporting animal above substrate; g, *in situ* view of *Democrinus* sp. between 200 and 300 m depth, off Discovery Bay, Jamaica. Total vertical height of animal 0.33 m.

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Archaeocyathid-bearing Erratics from Dwyka Subgroup (Permo-Carboniferous) of South Africa, and their Importance to Continental Drift

THE Dwyka Subgroup, forming the basal division of the Karroo Group, comprises a thick sequence of glacial morainic debris and boulder shales, together with subordinate shales, varvites, and lenticular sandstones. In the southern Cape, tillitic rocks are overlain by the so-called "Upper Dwyka shales", an argillaceous sequence at the top of which are white-weathering carbonaceous shales, the "White Band". The latter is overlain by a thin but persistent chert band which forms the arbitrary boundary with the overlying Ecca Subgroup. The Upper Dwyka shales have yielded all the animal fossils of this subgroup in the southern Cape. McLachlan and Anderson¹ have recorded orthocerid nautiloids, the brachiopod *Attenuatella*, the bivalves *Phestia* and (?) *Nuculopsis*, palaeoniscoid fish, radiolarians, spiral coprolites suggestive of the presence of sharks, fossil wood, foraminifers, and miospores from the base of the succession near Kimberley. They² favoured a Sakmarian age for this marine incursion. The non-marine³ White Band has yielded the aquatic reptile *Mesosaurus* and the crustaceans *Notocaris*, "Pygaspis", and *Anthrapalaemon*.

Glaciomarine beds spanning some 250 m at the base of the Dwyka succession in South West Africa³⁻⁵ have yielded the ammonite *Eoasianites* (*Glaphyrites*), an orthoconic nautiloid, possibly *Dolorthoceras*, the bivalves "*Aphanaia*" and *Eurydesma*, the gasteropod *Peruvipsira*, the coelenterate *Conularia*, the echinoid *Archaeocidaris*, the ectoproct *Dyscritella*, the palaeoniscoid fish *Namaichthys* and *Acrolepis*, a monasterid starfish, as well as crinoid stems, an indeterminate brachiopod, foraminifers, radiolarians, and fossil wood. *Mesosaurus*, as in the southern Cape, occurs only in white-weathering shales at the top of Dwyka succession^{3,6}.

In view of the relative scarcity of fossils within the strata of this Subgroup, it is of great interest to record the discovery of fossiliferous glacial erratics from the Prince Albert district. This find by one of us (R. O.) is all the more significant for

the fact that the fossils are Cambrian archaeocyathids (Fig. 1). They occur in grey limestone cobbles, representing transported glacial erratics, sporadically embedded in the tillites of the Dwyka Subgroup.

Archaeocyathids have a cosmopolitan distribution in Lower and Middle Cambrian marine deposits, during which period they built extensive reefs in Australia, North America, Europe, North Africa, Asia, and Antarctica. Nowhere are they known from younger deposits and the phylum is a short-lived one.

No marine rocks of Cambrian age are known from southern Africa. In South West Africa the Nama Group is of terminal Precambrian age⁷, characterised by an "Ediacaran" faunal element. Among the fossil genera recorded are *Rangia*, *Nasepia*, *Orthogonium*, *Pteridinium*, *Cyclomedusa*, *Ernietta*, and so on. Unconformably overlying the Nama Group are the "red-bed" deposits of the Fish River Formation, formerly included within the Nama Group⁸, but now considered to represent molasse deposition following the Damaran orogeny⁷. Martin⁸ considers the most



Fig. 1 Archaeocyathids from limestone cobbles embedded in the Dwyka tillite, approximately $\times 5$.

reliable age for the post-tectonic pegmatites intrusive into the Damara Group to be 510 ± 60 Myr. It seems probable, therefore, that the Fish River Formation spans at least part of the Cambrian period, a suggestion supported by Germs⁷ record of *Phycodes pedum* Seilacher from these sediments. No undoubted marine fossils are known from these predominantly terrestrial red-beds, and they thus provide an important palaeoclimatic marker for the Cambrian period in southern Africa.

In the north-western Cape, at Vanrhynsdorp, the Table Mountain Subgroup rests unconformably upon Nama strata, while in the southern Cape it is conformably succeeded by the marine strata of the Bokkeveld Subgroup, dated at lower Devonian (early Emsian)⁹. The recent assignment of an Upper Ordovician age (Upper Ashgill)¹⁰ to a brachiopod assemblage from the Cedarberg Formation suggests that the orthoquartzites of the Nardouw Formation themselves span the entire Silurian period, as well as the base of the Devonian (Fig. 2). An Upper Cambrian age for the basal beds of the Table Mountain Subgroup thus seems likely, and is supported by the red-bed deposits of the basal Graafwater Formation, in the absence of the conglomerates of the imper-sistent Piekenier Formation.

The Table Mountain Subgroup is separated by a regional disconformity from the underlying Klipheuwel Formation¹¹

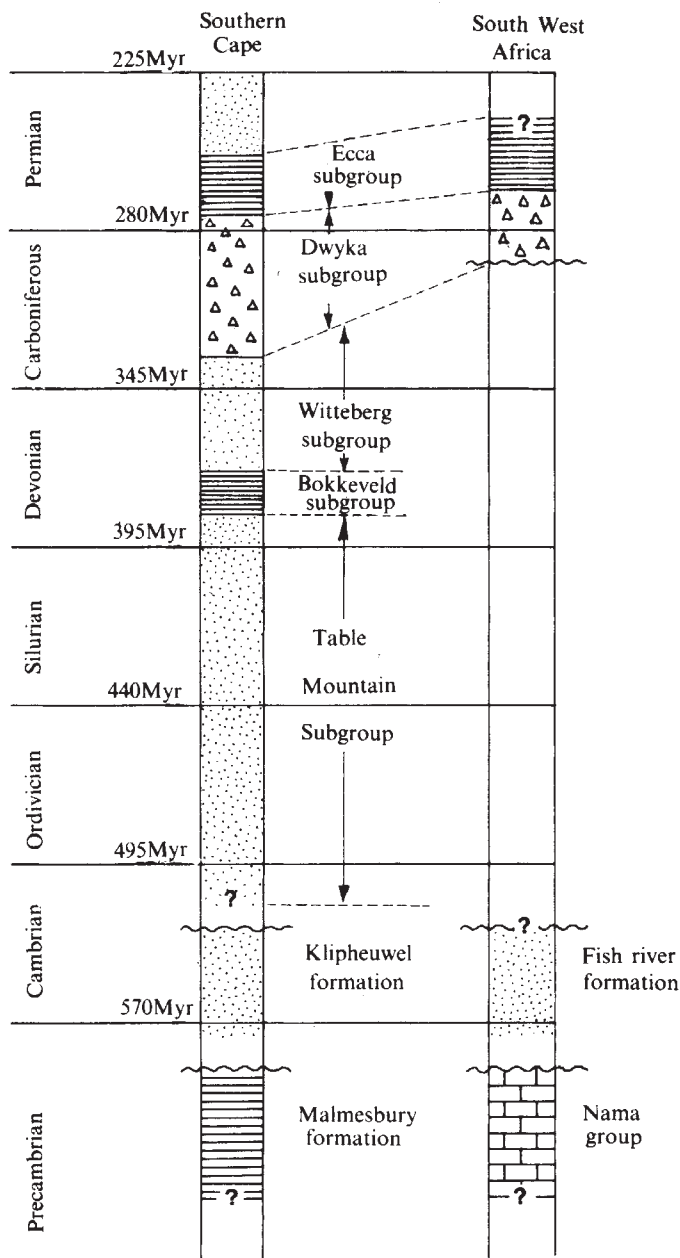


Fig. 2 The Palaeozoic chronostratigraphy of southern Africa.

in the southern Cape, the latter comprising brightly coloured greywackes and shales, and representing typical red-bed sediments. These sediments represent the lithostratigraphic, and in all probability, the chronostratigraphic equivalents of the Fish River Formation. Unfortunately they have yielded no fossils.

A study of the Cambrian deposits of southern Africa shows deposition to have been affected under terrestrial red-bed conditions, and thus most certainly not a source for wholly marine archaeocyathids. Where did these fossils come from? Their occurrence in glacial erratics suggests transport from a distant source area. It is necessary, therefore, to consider the palaeo-ice-flow directions of the Dwyka tillite.

Stratten¹², from a study of till fabric and glacial striae, considered the glacial deposits of the Prince Albert area (Fig. 3) to have been derived from the west (the Atlantic Ice Sheet), the north (the Namaland and Transvaal Ice Sheets), and the south (the Southern Cape Ice Sheet). Of these, the northerly

sources can be excluded since, as just shown, no marine Cambrian is known from southern Africa. Recent evidence¹³ suggests that the westerly source, that is, the Atlantic Ice Sheet, is incorrect, and that the glacial ice-flow direction was actually towards the west, thereby corresponding with known

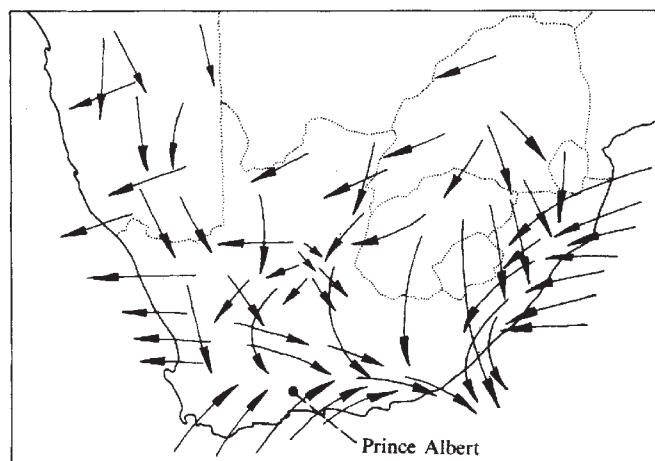


Fig. 3 Dwyka ice-flow directions. After Stratten¹² and Theron and Blignault¹³.

palaeo-ice-flow directions in South America. This leaves only the Southern Cape Ice Sheet as the transporting agent. On the Gondwana reconstruction (Fig. 4) this ice flow had its origin in Antarctica. It is of extreme interest, therefore, not only to note the occurrence of *in situ* archaeocyathid limestones in the southern Argentina Range and from the Nimrod Glacier—Beaumont Bay region of southern Victoria Land¹⁶, but also their occurrence in moraine boulders at Mount Spann¹⁷.

Thus the only feasible source for the archaeocyathids embedded within the tillites of the Dwyka Subgroup seems to be Antarctica, thereby providing unequivocal evidence for the former unity of the southern continents.

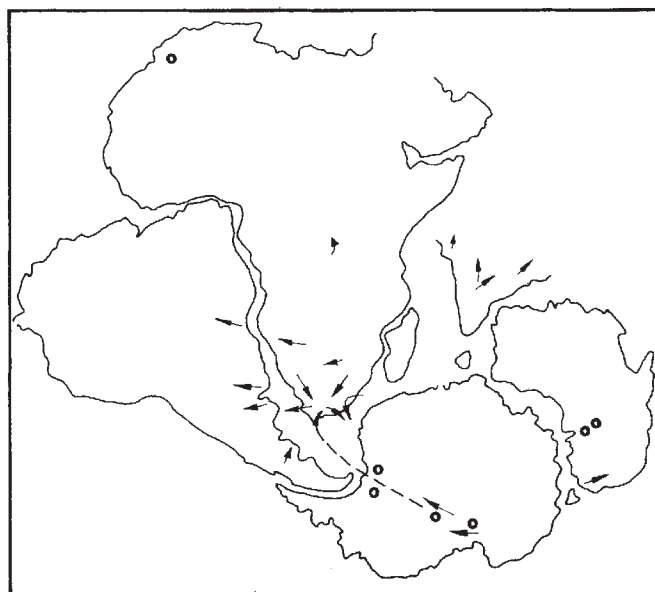


Fig. 4 The Gondwana distribution of archaeocyathids (circles), together with the late Palaeozoic ice-flow directions. After Stratten¹², Theron and Blignault¹³, Ahmad¹⁴, and Lindsay¹⁵.

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Discrimination learning in ascorbic acid-deficient guinea pigs

PAULING¹ has suggested that a high intake of vitamin C may be necessary for optimum cerebral function including learning and memory. We have tested this hypothesis in a preliminary way by comparing maze learning in two groups of guinea pigs: those receiving large supplements of ascorbic acid (the 'control' group) and those receiving a daily quantity of the vitamin sufficient to maintain a concentration in the brain at approximately 25% that of the controls (the 'deficient' group).

Thirteen male guinea pigs were reared on a diet well supplemented with ascorbic acid. When the animals were 9 weeks old (day 0) they were randomly assigned to one of two groups ('control' or 'deficient') and given an ascorbic acid-free diet². Drinking water was changed daily. That of the 'controls' contained 1% ascorbic acid throughout the experiment. From day 16, both groups received a daily oral dose of ascorbic acid (6 mg kg⁻¹ body weight) just sufficient to maintain good growth in deficient animals^{3,4}. Water consumption measurements indicated that controls received at least 500 mg kg⁻¹ body weight of ascorbic acid daily. This intake is much in excess of that required to saturate guinea pig tissues with vitamin C⁵.

From day 46 to day 67 animals were tested for spatial discrimination in a water T-maze (J. L. Smart, in preparation). On the first two days (days 46 and 47) escape was possible from both arms and animals were given five trials a day to test for left or right preferences. There followed 20 successive days of six trials per day during which escape was possible from only one arm of the maze. Animals were required to reverse previous preferred or learned responses. A learning criterion (successful 'reversal') was said to have been met when no errors (entries into arms other than the escape arm) were recorded during the last five of the six trials. The day after meeting this criterion the escape route was reversed.

TABLE 1 Body weight, tissue ascorbic acid and maze performance in control (saturated) and chronically ascorbic acid-deficient guinea pigs

		Control (n = 6)	Deficient (n = 7)
Body weight (g)	day 0	413 ± 61	420 ± 63†
	day 68	651 ± 40	626 ± 62†
Tissue ascorbic acid on day 68 (μmol g ⁻¹ wet wt)	Forebrain	1.11 ± 0.05	0.30 ± 0.05*
	Brainstem	0.70 ± 0.05	0.20 ± 0.04*
	Liver	1.67 ± 0.20	0.28 ± 0.17*
	Adrenals	6.49 ± 1.28	0.91 ± 0.15*
T-Maze performance (days 48 to 67)	Total reversals	7.0 ± 1.1	6.1 ± 2.9†
	Total errors	50.0 ± 11.0	48.7 ± 13.4†

Results are mean ± s.d.

* $P < 0.001$.

† Not significant (Student's *t* test) when compared with corresponding control value.

On day 68 animals were killed by decapitation and ascorbic acid was determined⁵ in brain, liver and adrenal glands.

In a parallel experiment mean forebrain ascorbic acid in deficient animals was, on day 15, $0.44 \pm 0.05 \mu\text{mol g}^{-1}$ ($n = 4$), and, on day 42, $0.26 \pm 0.25 \mu\text{mol g}^{-1}$ ($n = 4$). These values represent deficits, compared with controls (Table 1), of 60% and 77%, respectively. Brainstem showed deficits of 69% at 15 d and 63% at 42 d. On day 68 (Table 1) the deficits were 73% in forebrain, 71% in brainstem, 83% in liver and 86% in adrenals. It can be inferred that the brains of the deficient group were severely lacking in ascorbic acid both during maze learning and for at least 30 d before.

The chronic, severe brain vitamin C deficiency had no effect on T-maze performance, as assessed by either reversals or errors during 20 d of testing (Table 1). This indicates that learning is not enhanced by having saturating levels of ascorbic acid in the brain.

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Similarity of morphine abstinence signs to thermoregulatory behaviour

THE precipitated abstinence syndrome consists of a series of behavioural events which appear in morphine-dependent organisms after the administration of narcotic antagonists. Martin¹ has suggested that some precipitated abstinence signs arise because an error force is generated when a narcotic

antagonist resensitises a homeostat previously altered by a narcotic. Recently, in studies on the neuroanatomical correlates of morphine withdrawal, we found that brain areas associated with the wet shake behaviour of precipitated abstinence in the rat seemed to be closely adjacent to central pathways of heat dissipation and heat gain²⁻⁴. As morphine has complex effects on central thermoregulatory mechanisms⁵, the possibility was considered that the generation of an error signal in central thermoregulatory systems may account for the appearance of some precipitated abstinence signs. To test this hypothesis, we investigated the effects of environmental temperature on the precipitated abstinence syndrome.

Male Sprague-Dawley rats, obtained from Horton Laboratories (Oakland, California) and weighing 235 ± 21 g (s.d.) each, were used throughout these experiments. To produce dependence, two morphine pellets, each containing 75 mg of morphine base⁶, were implanted subcutaneously in the lower

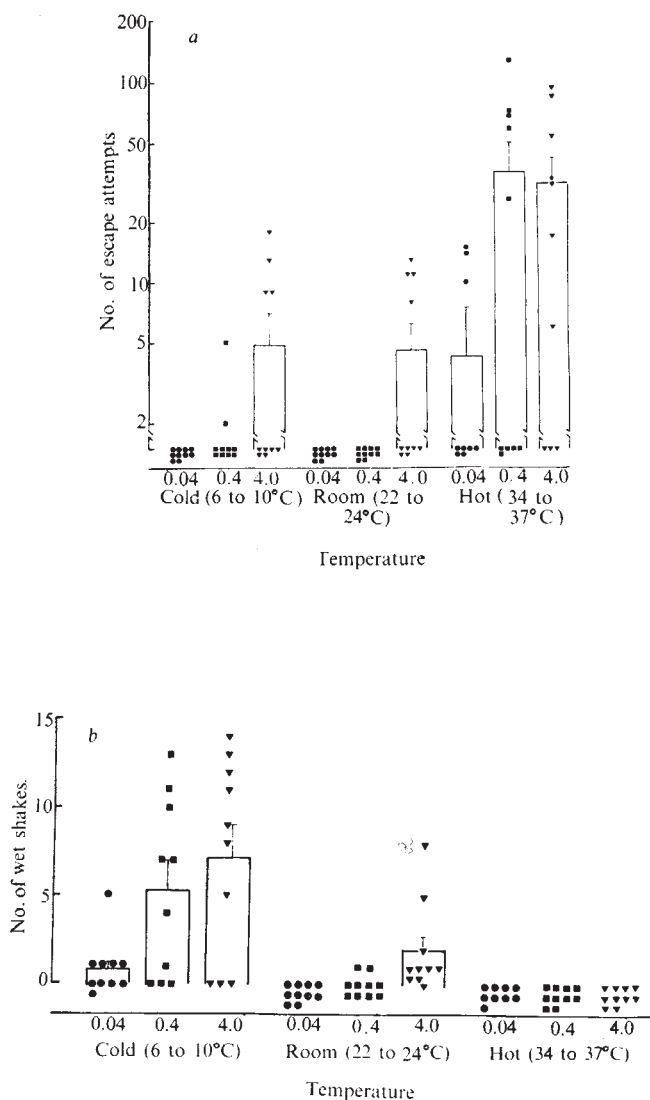


Fig. 1 Abstinence behaviour at different ambient temperatures. *a*, Escape attempts; *b*, wet shakes. Two pellets containing 75 mg of morphine base were implanted 48 h apart in male Sprague-Dawley rats weighing 235 ± 21 g (S.D.). Three days after the first pellet withdrawal was precipitated at different ambient temperatures by injecting naloxone hydrochloride at 0.04, 0.4, or 4.0 mg kg⁻¹ intraperitoneally. Withdrawal behaviour was observed for 10 min after naloxone administration. Each point represents the datum from one rat.

abdominal wall. The pellets of morphine were implanted 48 h apart. Twenty-four hours after the last pellet implant animals were weighed, placed in glass jars and then randomized into three groups. One group was kept at room temperature (22 to 24°C). The other groups were transferred to a hot (34 to 37°C) or cold (6 to 10°C) room. After 1 h the abstinence syndrome was precipitated in these different ambient temperatures by injecting naloxone hydrochloride intraperitoneally. (Endo Laboratories, Garden City, New York). Withdrawal behaviour was observed continuously for 10 min, as previously described elsewhere^{7,8}.

Animals placed in jars at the three different temperatures did not manifest any shaking behaviour or escape attempts. After 1 h in a hot environment, the ears and tails of the rats became red, the animal lay on its side with an extended posture and some salivation was observed. These signs have been reported as being characteristic behaviour of rats acutely exposed to a hot environment⁹. Animals kept in the cold, on the other hand, maintained a huddled position, the ears were blanched, and some degree of ptosis was observed.

As expected, the injection of naloxone in morphine-dependent rats kept at room temperature provoked the abstinence signs of wet shakes and escape behaviour^{7,8}. The effect of a cold or hot environment on these withdrawal signs was dramatic. Escape behaviour, precipitated by naloxone in a hot environment, was markedly enhanced: jumping was elicited in some animals with a dose of naloxone as low as 0.04 mg kg⁻¹ and at 4 mg kg⁻¹ most animals repeatedly jumped out of the jars as fast as the observer was able to put them back into the container (Fig. 1*a*). The most active rat jumped out of its jar 132 times within a 10 min period. By contrast, wet shakes behaviour was completely suppressed in the hot environment (Fig. 1*b*). In the cold environment, the frequency of wet shakes was enhanced but no change in the escape attempt frequency was noted (Fig. 1*b*).

As a hot or cold environment will trigger vascular adjustments and alter metabolic rate, it could be argued that the behavioural effects of temperature were secondary to changes in the distribution of naloxone to the brain. The results in Table 1 indicate, however, that a change in ambient temperatures does not significantly affect, within the 10 min observation period, the distribution of naloxone in serum or brain.

The results of this study demonstrate that the occurrence of certain withdrawal signs, wet shakes and escape attempts, can be markedly influenced by environmental temperature. In this context, one may ask: what is the physiological significance of wet shakes and escape attempts in the behavioural repertoire of the normal rat and, why are these withdrawal signs magnified or suppressed by environmental temperature?

TABLE 1 Effect of temperature on the distribution of naloxone in serum in brain

Treatment	Naloxone levels		
	Serum (μ g ml ⁻¹)	Brain (μ g g ⁻¹)	Brain/serum
Placebo (22 to 24°C)	1.38 $\pm$ 0.09	0.76 $\pm$ 0.05	0.55
Morphine (22 to 24°C)	1.48 $\pm$ 0.21	0.69 $\pm$ 0.21	0.47
Morphine (6 to 10°C)	1.54 $\pm$ 0.16	0.86 $\pm$ 0.15	0.56
Morphine (34 to 37°C)	1.35 $\pm$ 0.25	0.76 $\pm$ 0.18	0.56

Rats were implanted with two placebo pellets or two pellets containing 75 mg of morphine base. The second pellet was implanted 48 h after the first pellet. Three days after the first pellet, two groups were transferred for 1 h to a cold (6 to 10°C) or hot (34 to 37°C) environment. Naloxone hydrochloride, at a specific activity of 1.9 μ Ci mg⁻¹, was administered 4 mg kg⁻¹, intraperitoneally, and 10 min later, serum and brain tissues were obtained and measured for naloxone. Five animals were in each group. Values represent the mean $\pm$ s.e. No significant differences were observed in the different groups.

Hainsworth⁹ has observed that normal rats placed in a 40°C environment for 2 h or a 44°C environment for 1 h will try to escape from its cage by biting at any opening, by jumping up the side of the cage or by trying to push the top off the cage. By contrast, normal rats placed in extreme cold will demonstrate wet shakes. For example, cold water, wetness, and irritation to tissue around the ears will provoke this shaking behaviour⁴. It seems, therefore, that escape attempts and wet shakes, in certain experimental conditions, are forms of adaptive behaviour which enables the rat to maintain normal body temperature. Escape attempts could be a heat loss mechanism: the animal 'feels too warm' and attempts to escape from its environment. Wet shakes could be a heat gain mechanism similar to shivering.

Body temperature fluctuates widely during withdrawal from morphine¹⁰⁻¹². Some of the signs of abstinence in man and in experimental animals may reflect this disruption of thermoregulation. For example, shivering, gooseflesh, sensations of cold and warmth, profuse sweating, rhinorrhoea and lacrimation are characteristic withdrawal signs in man¹³. In the rat, during precipitated withdrawal at room temperature, teeth chattering, wet shakes, vasoconstriction, ptosis and a huddled posture may be observed⁷. This behaviour may reflect activation of heat gain mechanisms. Other withdrawal signs are salivation, the spreading of saliva on the body and escape behaviour. These latter signs may be manifestations of heat loss mechanisms⁹.

In recent studies we have presented data indicating that naloxone acts principally in medial mesodiencephalic regions of the brain to precipitate the abstinence syndrome²⁻⁴. The medial mesodiencephalon has traditionally been considered to be an important brain area for the integration of thermosensory information and for the regulation of body temperature¹⁴. Thus, from an anatomical viewpoint, it would seem plausible that certain precipitated withdrawal signs resemble a derangement of central thermoregulatory mechanisms. Escape behaviour and wet shakes may be viewed as behavioural events triggered by the sudden occurrence of an error signal in the thermosensory or thermal control system: compensatory heat gain and heat loss mechanisms are then activated to return the set-point to normality.

From these considerations, a tentative generalisation may be proposed for interpreting the effects of some drugs which modify the withdrawal signs of wet shakes or escape attempts. It is proposed that drugs which make the animal 'feel hot' will increase the frequency of escape behaviour and, possibly, suppress wet shakes whereas drugs which make the animal 'feel cold' will enhance the frequency of wet shakes without affecting escape behaviour. As pharmacologic agents frequently affect more than one component of the thermoregulatory system^{15,16}, considerable caution should be exercised in the interpretation of drug effects on morphine dependence¹⁷. The results of this study also indicate that it would be prudent to monitor environmental temperature during the observation of the withdrawal syndrome.

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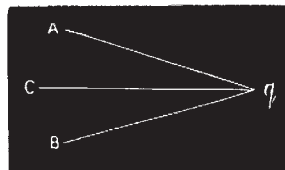
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Heaviside Radiation

It is now well known that a charged particle, moving through a refracting medium at a speed in excess of the phase velocity of electromagnetic waves, produces radiation. The effect is observed with energetic electrons in water and other media, from cosmic ray particles moving through the atmosphere and may also be a source of very low frequency radio waves in the ionosphere and magnetosphere. Its discovery is usually attributed to Čerenkov¹ and its theoretical interpretation to Frank and Tamm², all three of whom were jointly awarded a Nobel Prize in 1958.

Returning to the case of a charge q at a point moving through a dielectric, if the speed of motion exceeds that of light, the disturbances are wholly left behind the charge, and are confined within a cone, AqB. The charge is at the apex, moving from left to right along Cq. The semi-angle, θ , of the cone, or the angle AqC, is given by



$$\sin \theta = v/u,$$

where v is the speed of light, and u that of the charge. The magnetic lines are circles round the axis, or line of motion. The displacement is away from q , of course, and of total amount q , but not uniformly distributed within the cone. The electric current is towards q in the inner part of the cone, and away from q in the outer.

It will be seen that the electric stress tends to pull the charge back. Therefore, applied force on q in direction Cq is required to keep up the motion. Its activity is accounted for by the continuous addition at a uniform rate which is being made to the electric and magnetic energies at q . For the motion at the wave-front, at any point on Aq or Bq, is perpendicularly outward, not towards q . Whilst the cone is thus expanding all over, the forward motion of q continually renews the apex, and keeps the shape unchanged.

Steady motion alone is assumed.

To avoid misconception I should remark that this is not in any way an account of what would happen if a charge were impelled to move through the ether at a speed several times that of light, about which I know nothing; but an account of what would happen if Maxwell's theory of the dielectric kept true under the circumstances, and if I have not misinterpreted it. [See footnote on p. 516, later.]

Nov. 18, 1888.

Fig. 1

In view of the extract shown in Fig. 1 (ref. 3), may I suggest that the name of the British scientist, Oliver Heaviside, should be associated with this phenomenon.

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Heaviside-Mallet Radiation?

FOR those who have worked on Čerenkov radiation it is of great interest to read that Kaiser¹ has unearthed a prediction of the effect by Heaviside that predates Čerenkov's early work by 46 years, and it is remarkable that apparently, this has not been pointed out before.

It would seem, however, at least from the extract quoted by Kaiser, that Heaviside did not suggest or carry out any experiments. Indeed this was virtually impossible at that time, as his paper predates the discoveries of cosmic rays and radioactivity, and there were, of course, no beams of artificially accelerated relativistic particles available at that time.

It is therefore on the theoretical side that Heaviside's work is significant and his name should thus be linked with those of Vavilov² and of Frank and Tamm³.

Here I point out that, as far as the experimental side is concerned, it would seem rather that the credit should go to the French physicist Mallet, who, through his observations between 1926 and 1928 (refs. 4 to 6), was probably the first to study the effect always attributed to Čerenkov, a point already raised by myself⁷, by Mallet himself⁸ and by Perrin⁹. I therefore suggest that, for those who feel that changing established names for well known phenomena is justifiable, the title Heaviside-Mallet radiation might be more appropriate than the one put forward by Kaiser. In connection with double-barrelled titles it is of interest to note that for many years the effect has also been known as Vavilov-Čerenkov radiation, at least within the Soviet Union.

It is not inappropriate that a French name should be associated with the effect since there is little doubt that the glow from the concentrated radioactive solutions prepared by Mme Curie¹⁰ was generated by the same mechanism; her observations took place around 1910.

This letter should not, however, be interpreted as discrediting three brilliant Nobel Prize winners, for by far the greater proportion of all theoretical work in the field has nevertheless been carried out by Russian physicists. There are sixty-two Russian references in ref. 7 alone and two other relevant sources of information, both Russian, are the treatises by Bolotovskii¹¹ and by Zrelov¹².

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Alternative to Holography for Determining Phase from Image Intensity Measurements in Optics

IN light optics and electron optics one normally measures the image intensity and attempts to infer from these measurements the object structure. In light optics these intensity measurements are insufficient to evaluate the detailed structure of the object and information on the phase of the transmitted light is of prime importance¹; the interaction of the light or electrons in transmission through the object can be only satisfactorily explained by wave optics. The phase problem in optics is analogous to the X-ray diffraction phase problem, that is, whereas an object can be reconstructed from the phase information (even with incorrect amplitudes), it is not possible to define an object from amplitude measurements only¹. We suggest here how a normal optical system can be used to determine both the amplitude and phase from image intensity measurements in bright-field optics. In bright-field optics, where the main (on-axis) beam is allowed to interfere with the scattered wave, the transmitted object wavefunction $\psi_o(r_o)$ can be written as

$$\psi_o(r_o) = 1 + \psi_s(r_o) \quad (1)$$

where $\psi_s(r_o)$ represents the effect of the object on the incident light or electron wave at the point $r_o = (x_o, y_o)$ in the object plane; ψ_s carries information not only on the amplitude attenuation of the wave but also phase shifts introduced by refractive index differences (potential differences in electron optics) across the object. The 1 represents the amplitude of the transmitted wave that is not scattered in the object, and this unscattered contribution will give a background in the image. The wavefunction $\psi_o(r_o)$ transmitted by the object may be subsequently affected by lens defects and restricting apertures in the optical system, particularly in electron optics, but for simplicity we shall assume that the wave at the image plane $\psi_i(r_i)$ is equivalent to $\psi_o(r_o)$. We record not $\psi_i(r_i)$ but the image intensity $|\psi_i(r_i)|^2 \equiv |\psi_o(r_o)|^2$; the image intensity is from equation (1)

$$j(r_i) = |\psi_i(r_i)|^2 = |1 + \psi_s(r_i)|^2 \\ = 1 + 2\text{Re}[\psi_s(r_i)] + \text{Re}^2[\psi_s(r_i)] + \text{Im}^2[\psi_s(r_i)] \quad (2)$$

where Re and Im denote the real and imaginary parts of ψ_s . At best, if the interaction between the light or electron wave and the object is small and the squared terms in equation (2) can be neglected, we can only determine the real part of ψ_s from image intensity measurements, and there is no direct way of evaluating the imaginary part of ψ_s , hence determining both the amplitude and phase. But instead of using a normal circular aperture in the back focal plane (Fourier plane) of the objective lens, we can use a semicircular (half-plane) aperture to cut off one-half of the Fourier plane of the object wavefunction. The Fourier plane, which corresponds to the diffraction or scattering plane, displays the spatial frequencies

of the object structure, and the idea is to eliminate initially one-half of the object frequencies from the image. Now there is an explicit relationship between the real and imaginary parts of ψ_s . We maintain the bright-field situation by having a small centre section cut out from the aperture to allow the main beam through to interfere with the scattered wave—this is strictly necessary in electron optics to avoid electrical

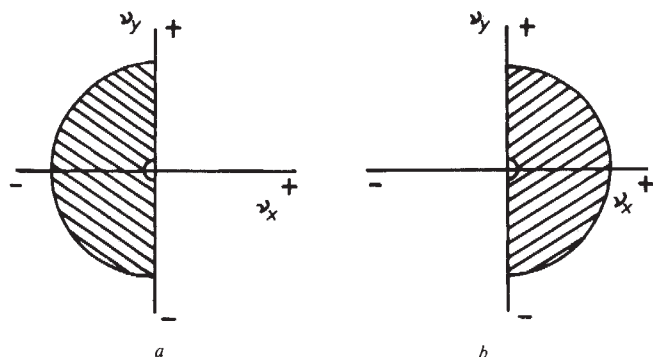


Fig. 1 Semicircular aperture positioned in the back focal (Fourier) plane of the objective lens. *a*, Negative half of plane excluded; *b*, positive half of plane excluded by the aperture.

charging problems. Suppose we omit the negative half of the Fourier plane (spatial frequencies $\nu_x < 0$), but allow all positive frequencies to contribute to the image; then the image intensity corresponding to this situation (Fig. 1*a*) is, omitting subscripts *i*,

$$[j(r)]_{\nu_x > 0} = 1 + 2\text{Re}[\Psi_s(r)]_{\nu_x > 0} + \text{Re}^2[\Psi_s(r)]_{\nu_x > 0} + \text{Im}^2[\Psi_s(r)]_{\nu_x > 0} \quad (3)$$

The idea of using a half-plane aperture is not new², but the important relationship between $\text{Im}(\psi_s)_{\nu_x > 0}$ and $\text{Re}(\psi_s)_{\nu_x > 0}$ has not been given. The relationship between the real and imaginary parts of $(\psi_s)_{\nu_x > 0}$ can be derived from standard Fourier theory³ and the analyticity of ψ_s ⁴, and in order to avoid detailed mathematics, we state the result that the imaginary part of $(\psi_s)_{\nu_x > 0}$ is a one-dimensional Hilbert transform of the real part; that is,

$$\text{Im}[\psi_s(x, y)]_{\nu_x > 0} = \frac{1}{\pi} \int_{-\infty}^{+\infty} \frac{\text{Re}[\psi_s(z, y)]_{\nu_x > 0}}{z - x} dz \quad (4)$$

taking the Cauchy principal value of the integral. From an image recorded using a semicircular or half-plane aperture we have an explicit relation between $\text{Re}(\psi_s)_{\nu_x > 0}$ and $\text{Im}(\psi_s)_{\nu_x > 0}$. But the squared terms in equation (3) prevent a direct calculation of $\text{Re}(\psi_s)_{\nu_x > 0}$ from the image intensity $j_{\nu_x > 0}$. We have solved this problem by initially neglecting these squared terms and we calculate $\text{Re}(\psi_s)_{\nu_x > 0}$ from $1/2(j_{\nu_x > 0} - 1)$; using this approximation to $\text{Re}(\psi_s)_{\nu_x > 0}$ we evaluate $\text{Im}(\psi_s)_{\nu_x > 0}$ using a form of equation (4) which avoids the problem of the singularity at $z = x$ and also takes account of the finite extent of the actual image; again this is a mathematical detail. We now have values for both the real and imaginary parts of $(\psi_s)_{\nu_x > 0}$ and a correction is applied to equation (3) for the squared terms, $\text{Re}^2 + \text{Im}^2$; it may be necessary to apply this correction several times, depending on the relative contributions of the squared and linear terms to the image intensity $j_{\nu_x > 0}$. In numerical tests, contributions from these squared terms of 25% of the value of $j_{\nu_x > 0}$ can be systematically corrected in 1–5 iterations. This magnitude for the correction is well within the normal conditions of electron microscopy, where image contrast is only 20% (of which squared terms contribute 10%); $\text{Re}^2 + \text{Im}^2 = 0.25$ (relative to the unity of the unscattered beam) would correspond to a very thick object with a strong interaction

between the incident wave and the object, corresponding to about 25% scattering from the incident beam.

By using such a half-plane aperture we have excluded all the information from the negative spatial frequencies ($\nu_x < 0$) and the result for just $(\psi_s)_{\nu_x > 0}$ is not a complete representation of the object ψ_s (including all spatial frequencies). We avoid this limitation by using a complementary semicircular aperture (Fig. 1*b*) or by just inverting the first half-plane aperture, excluding the $\nu_x > 0$ half of the Fourier plane, to give an equation corresponding to (3) with $\nu_x < 0$; the Hilbert transform relationship between $\text{Re}(\psi_s)_{\nu_x < 0}$ and $\text{Im}(\psi_s)_{\nu_x < 0}$ may again be derived, differing from (4) only in a change in sign on the right-hand side of the equation. Thus by recording two images with complementary semicircular (half-plane) apertures (Fig. 1) we can reconstruct from image intensity measurements the scattered wave immediately after the object ψ_s , by the addition of the two half-plane functions $(\psi_s)_{\nu_x > 0}$ and $(\psi_s)_{\nu_x < 0}$.

If the object structure has a centre of symmetry or a mirror plane we need only one image taken with a semicircular aperture to determine the complete object wavefunction ψ_s .

There are practical points to be considered if this method is to work; for example, the two component (half-plane) images of ψ_s , $(\psi_s)_{\nu_x > 0}$ and $(\psi_s)_{\nu_x < 0}$, have to be added to give ψ_s and the problem of correlating two images arises⁵. It is assumed that during the period of recording the two images the object does not change (for example, due to radiation damage in electron optics), that the objective lens focus does not change, that the two apertures are exactly complementary. The experimental arrangement seems fairly simple in light optics but the electron-optical equivalent, in which the sizes of apertures are scaled down to 50 μm , is subject to the problems of severe contamination and electrical charging of the half-plane aperture⁶.

The method outlined here for determining the amplitude and phase from image intensity measurements does not have the non-uniqueness problems associated with general iterative schemes for determining the phase^{7,8}, because we have a very good approximation to the real part of the wavefunction and we derive a value for the imaginary part which should at least place the phase angle ($\tan^{-1}(\text{Im}/\text{Re})$) in the correct quadrant. In bright-field optics we propose that the method of recording complementary half-plane images can be used to determine the amplitude and phase of the object wavefunction, without severe restrictions on the magnitude of the wavefunction, such as the phase grating approximation. The method does not work in dark-field optics, where the main (unscattered) beam is excluded from the image; this is because we can no longer derive an approximation to $\text{Re}(\psi_s)$ as in the bright-field case and the iterative solution of the Hilbert transform equation (4) is no longer unique.

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Acuity and contrast sensitivity of infant vision

If we are to study the development of form and space perception in man, it is necessary to know something of the basic visual capabilities of the developing human infant. There have been a number of investigations of visual acuity in the first few months of life. Using fixation preference as a measure, Fantz *et al.*¹ showed that infants could discriminate a stripe pattern of 1.5 cycles per degree by 1 month, and 3 cycles per degree by 2 months. Dayton *et al.*² found that newborn infants showed optokinetic nystagmus to a 4 cycles per degree pattern. These studies demonstrate a useful degree of visual spatial resolution in very young infants, though one markedly poorer than the typical adult value of 40 cycles per degree.

An observer's visual acuity is a measure of the highest spatial frequency (finest grating) that he can detect. Much more information about the spatial performance of the visual system is expressed by a contrast sensitivity function^{3,4}; that is, the threshold contrast for a range of sinusoidal gratings that vary in spatial frequency. We report here a preliminary attempt to determine the contrast sensitivity function of an infant, who was aged 50–62 d when the observations were made.

We used Teller's⁵ modification of the fixation preference technique. The infant was held in front of a display with two windows. On each trial one window contained a vertical grating while the other contained a uniform field of equal mean luminance. One experimenter observed the infant from behind the display, without knowing which window contained the grating. On the basis of 15 s observation of the infant's fixations and other behaviour, he had to guess on which side the grating was presented. For a given spatial frequency, the trials were run in blocks of five of constant grating contrast, the contrast being varied between blocks according to a modified staircase procedure⁶ (average length of staircase, 50 trials) until the value of contrast at which the observer gave 70% correct reports could be estimated. This value was taken as a 'threshold' contrast for that frequency. The procedure was then repeated for a different frequency. The validity of the technique is supported by the finding that the % of correct reports declined monotonically with decreasing contrast when frequency was held constant. For comparison, we also measured the contrast threshold of an adult observer in the same apparatus by the method of adjustment, at each frequency used.

The gratings were produced by the sinusoidal modulation of a high-frequency raster on a large oscilloscope screen³. The modulating oscillator was gated so that the grating appeared in one half of the screen only, that is, behind one of the windows, the unmodulated raster appearing behind the other. The grating was presented to left or right on successive trials according to a random sequence. Contrast was varied by passing the oscillator output through an attenuator. The mean luminance of both windows was 2.2 cd m⁻². At the infant's viewing distance (57 cm), the square windows subtended 7.5° each and their centres were separated by 18°.

Refractive examination of the infant showed that she required no spherical correction but showed some astigmatism (left eye +1D, 70°; right eye +0.5D, 90°). The ocular media and retina appeared normal. The stimuli were thus within the probable accommodative range of the infant⁷. It was quite possible, however, that she was not correctly accommodating; this adds to the grounds for regarding the obtained data as a lower limit on her discriminative capacities.

There were two types of presentation. In one, the gratings drifted across the window at 4.7 degrees s⁻¹, the direction of motion reversing every second. In the other, the modulation was stationary but was switched on and off ('flashed')

at 1.5 Hz, mean luminance remaining constant.

The results for the infant and adult observers, for both types of presentation, are plotted in Fig. 1. The infant's sensitivity is markedly depressed overall compared to the adult's; however, it should be remembered that we depended upon an observable preference to indicate the infant's detection, and so her results can be regarded only as giving a lower limit on her capacity for detection.

The infant's sensitivity functions show a number of features found in the adult. In particular, both infant and adult had a peak sensitivity for flashing stationary gratings at intermediate frequencies with a sharp high-frequency cut above 4 cycles per degree, and a decreasing sensitivity below 1 cycle per degree.

Both showed better performance with flashing than with drifting gratings at high spatial frequencies, and a more marked low-frequency cut with the flashing grating⁸. The extent of the low- and high-frequency cuts with the drifting display was presumably due to the use of a constant drift velocity (a temporal modulation rate proportional to spatial frequency). For the adult, the peak sensitivities for the two types of display were equal, but the infant showed greater overall sensitivity for flashing gratings. We cannot say whether this is due to a true difference in relative detectability, or to a more marked preference for flashing gratings than for drifting gratings of equal detectability.

Above 1 cycle per degree, the infant's curves are each depressed by a constant ratio of contrast relative to the adult's. This depression is greater for the drifting than for flashing gratings. At the low spatial frequencies, however, the

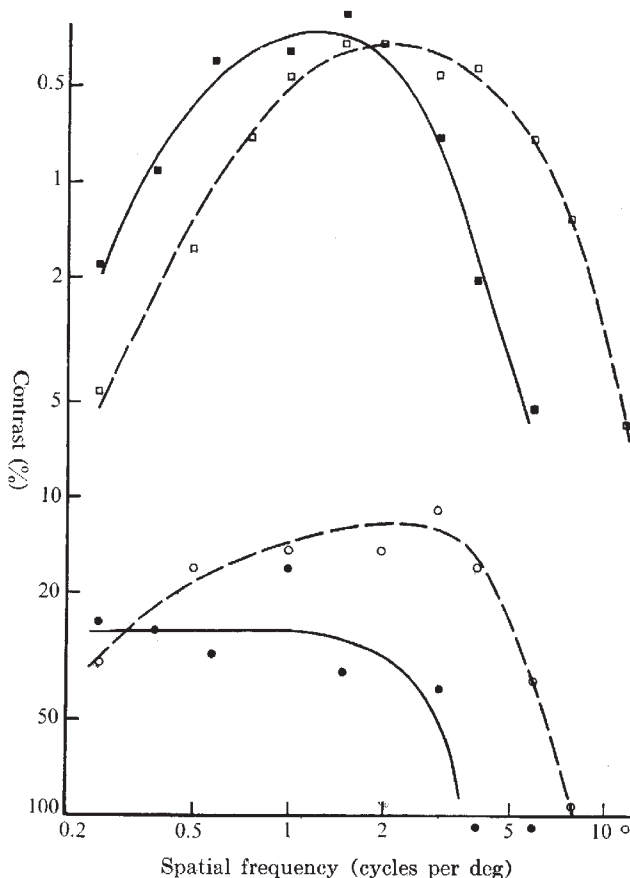


Fig. 1 Contrast sensitivity as a function of spatial frequency, for infant and adult observers (see text for methods of measurement). ■, □ Adult observer; ●, ○, infant observer; ○, □, flashing gratings; ●, ■, drifting gratings. Both axes are logarithmic, and the ordinate is plotted with contrast decreasing upwards. Points below the abscissa indicate values of spatial frequency for which discrimination did not reach the 70% level for 100% contrast.

gap narrows for both curves; the infant appeared relatively more sensitive in this region. Again, the difference can be explained in two ways. The visual pathway of the infant may be so organised as to transmit low spatial frequencies relatively well; for example, her visual neurones may have receptive fields whose inhibitory receptive fields are less developed than their excitatory centres. Alternatively, the difference may be due to a preference for low spatial frequencies over equally detectable high frequencies.

The point at which the curves cut the abscissa (100% contrast) represents visual acuity as conventionally measured with high-contrast targets. For the infant viewing stationary flashing gratings this point is at about 8 cycles per degree. This is a higher acuity than that previously found for infants 60 d old and younger. This can be attributed to the use of forced-choice by a 'blind' observer, which allows the use of more varied and subtle aspects of the infant's behaviour than simply timing fixations, and to the use of drifting and flashing targets, which are probably more attention-compelling to the infant than the static patterns used in earlier preference studies.

We thank Mr J. A. Movshon for advice and loan of the apparatus; Dr D. Y. Teller, whose unpublished manuscripts suggested the observation technique; and Miss J. M. Woodhouse for carrying out the refractive examination.

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dependent nystagmus, because standard theory³ holds that the two otolithic receptors are sensitive to gravity but cannot cause nystagmus, whereas the three semicircular canal receptors are not sensitive to gravity but do cause nystagmus. The mechanism whereby the vestibular receptors initiate PAN now seems obvious in the light of 'heavy water nystagmus', described here.

In ten male human subjects who had been fasting at least 10 h, we observed eye movements by using Frenzel spectacles and by using electrooculography² behind closed eyelids. Eye movements were observed with the subject's head in the supine position, with the left side down, and with the right side down, and the tests were repeated hourly between 30 min and 7½ h after drinking heavy water (deuterium oxide). The subjects weighed between 67 and 86 kg, and the dose was not adjusted for body weight; five subjects drank 200 g of deuterium oxide and five drank 100 g.

None of the subjects had spontaneous or positional nystagmus before drinking deuterium oxide, and one subject had none after drinking 100 g of it. In four of five subjects after drinking 100 g and in all five after drinking 200 g, there was vigorous positional nystagmus in the head—lateral positions (Fig. 1). When the head was held with the right side down, the nystagmus was to the left; when the head was held with the left side down, the nystagmus was to the right. These nystagmuses were present 30 min after drinking deuterium oxide and they could be elicited for a further 5 to 6 h. Heavy water nystagmus has directions opposite to PAN I.

Most of the subjects experienced strong sensations of bodily rotations, dizziness, and nausea while lying in the head—lateral positions. The nausea subsided slowly between tests, and the other effects disappeared a few seconds after

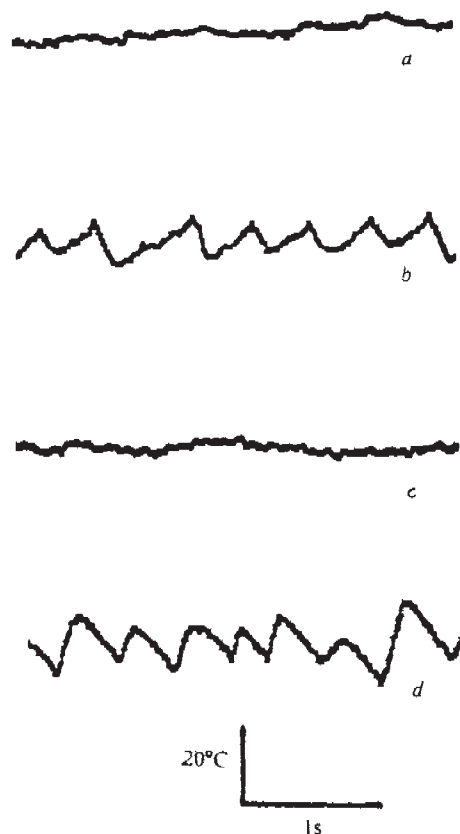


FIG. 1 Electronystagmographic traces of horizontal eye movements in a human subject with eyes closed. Pen movement upward indicates eye movement to the right. a, Lying right side down, before drinking D₂O; b, lying right side down, 30 min after drinking 200 g of D₂O; c, lying left side down, before drinking D₂O; d, lying left side down, 30 min after drinking 200 g of D₂O.

Heavy water nystagmus and effects of alcohol

AFTER drinking a large dose of ethyl alcohol (1 or 2 g of alcohol kg⁻¹ of body weight), a phenomenon called positional alcohol nystagmus (PAN) occurs^{1,2}. Nystagmus is an involuntary oscillating movement of the eyeballs in which they repeatedly turn slowly in one direction and fast in the reverse direction. The nystagmus that starts 30 min or so after drinking alcohol (PAN I) is called positional because it occurs only when the head is held in certain positions relative to gravity. When the head is held with the right side down, the eyes turn (with a frequency of roughly two complete oscillation s⁻¹) fast to the right, slowly to the left, fast to the right, and so on. Nystagmus is named after the prominent fast movements, and this nystagmus is called nystagmus 'to the right'. When the head is held with the left side down after alcohol, the resulting nystagmus is to the left.

It has been known for more than 40 yr (ref. 1) that PAN is caused, after ingestion of alcohol, by the action of gravity on the vestibular apparatus. In people or other animals who have lost the vestibular apparatus, the organ of balance, PAN never occurs. It was always thought puzzling that these vestibular sensory receptors could initiate a gravity-

assuming the normal upright posture. It was not surprising that the motion sickness syndrome appeared during the tests, as it is well known⁴ that unusual vestibular stimulation is the cause of motion sickness. There were no exceptions, in our limited sample of ten human subjects, to the generality that a subject's degree of nausea in the heavy water experiment varied inversely with his experience with alcohol ingestion. The heavy drinkers did not have nausea whereas the abstainers suffered greatly.

In a second experiment with four cats we demonstrated alcohol nystagmus and, after an interval of 1 week, we demonstrated heavy water nystagmus, using both electro-nystagmography in the dark and visual observation in the light. All of the cats after ingesting the deuterium oxide showed both the horizontal and rotary nystagmus, all with directions opposite to those found^{5,6} in the PAN I of cats. This result is consistent with the generality that heavy water nystagmus is precisely the opposite of PAN I. After a further interval of 1 week, heavy water and alcohol were administered in a mixture of the original doses of 4 ml of heavy water kg⁻¹ and 2 ml of alcohol kg⁻¹, to see whether the presumed opposite effects would cancel. In two of the cats a weak nystagmus of the heavy water type was discernible briefly, and in the other two cats no nystagmus occurred; this result was in contrast to the vigorous and prolonged nystagmus in all four cats after ingestion of either chemical alone.

The apparent mechanism whereby heavy water elicits nystagmus is very simple. The cupula of the semicircular canal has the same density as the liquid (endolymph) in which it floats. The cupula is normally moved by circular endolymph movements resulting from angular accelerations, and because of its neutral buoyancy the cupula normally is not moved by linear accelerations or by gravity^{7,8}. After ingestion of heavy water, the base of the cupula, because of its proximity to blood capillaries, acquires heavy water more rapidly than the surrounding endolymph. The cupula thereby loses its neutral buoyancy sufficiently⁸⁻¹⁰ so that gravity can move it.

Alcohol nystagmus can be similarly explained. In cats with various semicircular canals discretely inactivated it has been shown^{5,6} that, in PAN, gravity acts on the semicircular canals. This finding concerning the site of action of gravity has been independently confirmed¹¹, but it has not been explained how the canals become sensitive to gravity after alcohol ingestion. It now seems obvious that, just as heavy water can make the cupula heavier than endolymph until the heavy water diffuses well into endolymph, so alcohol which is lighter than water can make the cupula lighter than endolymph until the alcohol concentration in endolymph rises sufficiently.

A variety of implications follows from these findings and interpretations. The second phase^{12,13} of alcohol nystagmus (PAN II) probably results from the presence of alcohol in endolymph after the alcohol has largely disappeared from the cupula, and residual alcohol in the inner ear (with resultant symptoms of motion sickness) is probably a factor in the production of a hangover. In the clinical and experimental uses of heavy water for determination of total body water, the head—lateral orientations should be avoided because motion sickness and a profound antidiuresis¹⁴ can result.

Anyone who has assimilated heavy water, even as little as 45 ml through the lungs and skin, should avoid circumstances where bodily disequilibrium would be disastrous. Although it is a standard clinical view¹⁵ that non-fatiguing positional dizziness and nystagmus are usually caused by the action of gravity on the utricular otoliths (often in the presence of central pathology), it now seems possible that some of these conditions are caused simply by the cupula's loss of neutral buoyancy.

We thank the DCIEM toxicology group for labyrinthine and blood alcohol determinations, Al Nicholas, Ron Cardin, Jerry Laufer, and Rolf Roehrlé for techniques, Jack Landolt and Dick Malcolm for information and discussion.

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Temperature distribution inside a burning cigarette

THE study reported here has been undertaken to resolve the large discrepancies between reports of the temperature distribution in the combustion coal of a cigarette. Studies using bare thermocouples (refs 1-4 and R. G. Hook, paper presented at Twentieth Tobacco Chemists' Conference, Winston-Salem, North Carolina, November 1966) indicate that the highest temperatures occur on the central axis of the coal (the actual reported values vary considerably but are usually in the range 800-900° C during a puff, 700-800° C during the natural smoulder between puffs, and 800-850° C under steady state continuous draw conditions). Under all smoking regimes, the thermocouple-measured temperature decreases by up to 300° C along a radius from the maximum central temperature to the periphery of the coal, although one thermocouple study² has reported the occurrence of occasional peripheral hot spots, with temperatures higher than the centre of the coal. (Ref. 2 contains a comprehensive compilation of more than forty studies of temperature measurements inside burning cigarettes prior to 1968.) In contrast, temperature measurements by X-ray observations of the melting of small metal particles placed within the cigarette⁵ indicate that the highest temperatures (900° C) occur at the periphery of the coal. Furthermore, measurements of the coal's surface temperature by radiation methods (ref. 5 and A. T. Lendvay, F. M. Watson III, and T. S. Laszlo, paper presented at the CORESTA/Tobacco Chemists' Joint Conference, Williamsburg, Virginia, October 1972) have recorded temperatures of about 850-920° C during a puff, 700° C (below the surface ash) during smoulder, and short-lived hot spots on the coal's surface as high as 1200° C (ref. 5).

In all previous studies, no attempt has been made to define exactly which temperature is being measured in the heterogeneous coal—that of the solid matrix of the coal or that of the gases and smoke within the coal. Bare thermocouple junctions, and the metal particles of the X-ray study, would be in physical contact with the tobacco strands and so

would indicate a temperature intermediate between that of the gas and the solid phases, the actual temperature depending on the degree of strand-junction contact. On the other hand, radiation methods would indicate solely the temperature of the solid matrix.

In the present study, a quartz probe has been used to measure the gas- and solid-phase temperatures independently inside a cigarette. The probe contained a platinum/platinum-13% rhodium thermocouple junction (0.05 mm diameter wire) mounted at its end, and an infrared transmitting fibre optic (0.15 mm outer diameter, part of a Vanzetti 1074 thermal probe system). The fibre optic was sheathed with a metal tube for 6 mm to restrict its field of vision to directly ahead. Its viewing end was placed 12 mm behind the end of the quartz tube so that the delicate fibre optic was not subjected to the high ambient temperatures inside the coal. Non-filter English-type cigarettes (68 mm long, 8 mm diameter) were selected for weight (1.00 ± 0.02 g) and pressure drop (10.6 ± 0.3 mm water gauge, at a flow rate of $2.0 \text{ cm}^3 \text{ s}^{-1}$) and conditioned at 21°C and 60% relative humidity (1 mm water gauge = 9.8 N m^{-2}). The probe was inserted radially into the cigarette and the hole in the cigarette paper sealed with a minimum amount of latex solution. The cigarette was smoked at a constant draw of $2.0 \text{ cm}^3 \text{ s}^{-1}$. The temperature-distance relationships were determined at a fixed point, initially 20 mm from the lighted end of the cigarette, and distances of 0, 2, 3 and 4 mm from the central axis (4 mm is the surface measurement). The internal temperature profiles are the mean of four determinations whereas the external temperature profile is the mean of twenty determinations (because of the presence of variable surface hot spots).

The temperature distributions above 300°C are reported. Since smoke aerosol particles do not exist above about 300°C , the coal above this temperature is a gas-solid system. The contribution of radiation from heteropolar molecules to the overall infrared emission inside the coal is negligible. The temperature obtained from the radiation transmitted and detected by the fibre optic probe system ($1.2\text{--}1.6 \mu\text{m}$) is thus almost entirely that of the solid matrix of the coal (considered to be a blackbody). The thermocouple junction situated at the end of the quartz tube was positioned so that it was not in physical contact with tobacco strands, and so received heat from the gas phase by convective transfer and from the solid phase by radiation. Heat transfer calculations, using the solid phase temperatures obtained from

the infrared system, together with the thermocouple temperatures, gave the temperature distribution of the gas phase. The analysis indicated that heat exchange with the gas phase is the predominant mechanism by which the thermocouple junction receives heat.

By analysing temperature values obtained in different experiments with the probe inserted radially and axially to given positions inside the cigarette (and hence subject to different temperature gradients), an empirical correction has been applied to the present results to compensate for heat losses by conduction along the probe and thermocouple leads. This correction can be as high as 96°C in the peripheral region of the coal, where the temperature gradients are steepest.

The distributions obtained for the temperatures of the two phases are shown in Fig. 1. They are not alike, indicating that thermal equilibrium between the gas and solid phases has not been achieved. The mean maximum solid temperature (750°C) occurs on the surface of the coal, at a distance of 2.6 mm in front of the line of paper burn. In addition, small hot spots of diameter 0.1–0.8 mm and temperature $780\text{--}830^\circ \text{C}$ occur on the surface of the coal at variable distances of between 1 and 5 mm in front of the line of paper burn. Since an individual tobacco strand in the cigarette has a thickness and width of 0.1 and 0.5 mm respectively, these hot spots probably correspond to the behaviour of individual tobacco strands. For distances of less than 1 mm along any radius below the surface of the coal, the temperature of the solid decreases due to the occurrence of endothermic heterogeneous reduction of carbon dioxide by the carbonaceous coal, the occurrence of endothermic pyrolysis reactions and heat exchange with the cold gases entering the interior of the coal. At distances of more than 1 mm below the coal's surface (along any radius), the temperature of the solid phase starts to increase, and attains a temperature of $600\text{--}750^\circ \text{C}$ over a large proportion of the interior of the coal.

In contrast, the gas phase temperatures at the coal's surface are relatively cool ($\leq 400^\circ \text{C}$). Beneath the surface of the coal, and towards the cigarette central axis, the temperature of the gases continuously increases due to the occurrence of exothermic gas phase reaction (such as the oxidation of carbon monoxide) and heat exchange with the solid matrix. The maximum temperature of the gases in the coal (810°C) occurs on the central axis of the cigarette, 11.5 mm in front of the line of paper burn. In the interior regions of the coal (2 mm or more below the surface), the temperatures of the solid and gases are relatively close (to within 60°C), and dT/dr values for the two temperatures are similar and both have positive values (T = temperature, r = distance below surface of the coal). In this region of the coal, thermal equilibrium is closest to being attained.

This work has shown that, where heterogeneous reactions are occurring within porous beds, the phases involved can have widely differing temperatures, which may be determined by the techniques described.

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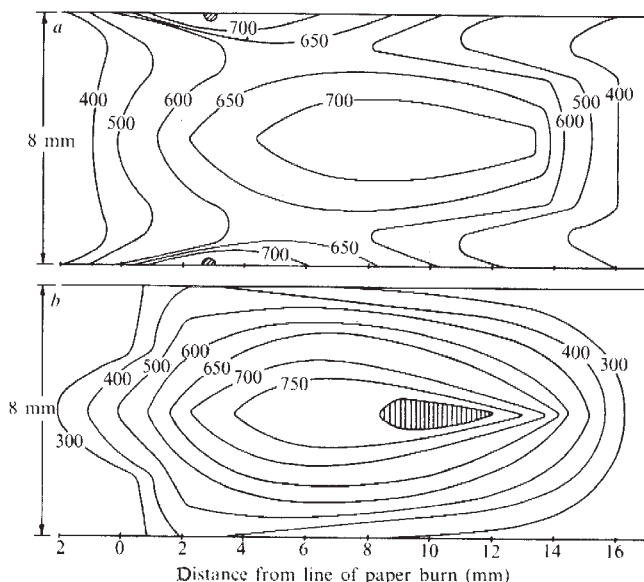


FIG. 1 Temperature distribution within the coal, with cigarette smoked at a constant draw of $2.0 \text{ cm}^3 \text{ s}^{-1}$. a, Temperature of solid (hatched area 750°C); b, temperature of gases (hatched area $>800^\circ \text{C}$).

book reviews

Inhabiting an island

Surtsey, Iceland: The Development of a New Fauna, 1963–1970: Terrestrial Invertebrates. By Carl L. Lindroth, Hugo Andersson, Högni Bödvarsson and Sigurdur H. Richter. Pp. 280. (Supplement 5 of *Entomologica Scandinavica*.) (Munksgaard: Copenhagen, October 1973.) 140 Dan. Cr.

THE volcanic island of Surtsey appeared in November 1963, had assumed its present form by July 1967, and is now the southwesternmost and second largest member of the Westman group. At the Surtsey Biology Conference in Reykjavik on May 28, 1965, it was decided to keep the progressive arrival of plants and animals on the new island under close observation: Dr Lindroth and his three colleagues were entrusted with the investigation of the terrestrial invertebrates.

The book is divided into three parts. In the first part the authors stress the advantages Surtsey has over similar new volcanic islands for this sort of study. They point out that the other small Westman islands were all formed by volcanic action about 6,000 years ago and probably acquired their fauna and flora stage by stage, in a way it is now possible to observe in detail on Surtsey, but that it may be centuries before the Surtsey biota reaches a climax comparable to that on the other islands.

In the second part of the book, the terrestrial invertebrates of Surtsey and its 'Hinterland' are listed, each with its local and general distribution and ecology. The term 'Hinterland' is used to include the other small Westman islands, Heimaey and the adjacent south coast of Iceland. Most of this part is devoted to exclusively Hinterland species but, as the authors explain, a knowledge of the numerous species which might have spread to Surtsey but have not yet done so is an essential part of their study. The 159 species actually recorded from Surtsey consist of 131 pterygote insects (of which 105 are Diptera), six Collembola, five spiders and seventeen mites.

In the third and largest part of the book, details of the frequency and abundance of the Surtsey species are given. Modes of dispersal, namely aerial (both active and passive), hydrochorous, zoochorous and anthropochorous, are discussed at some length. Precautions which are being taken to minimise anthropochorous dispersal and experiments to test the likelihood of hydro-

chorous dispersal are described. It is concluded that, although certain migratory Lepidoptera and one species of ballooning spider may have arrived direct from Europe or even North America, nearly all the species recorded from Surtsey have spread from the Hinterland. Most of the species require conditions for the completion of their life cycles which are not yet available on the island, largely because of the very slow spread of vascular plants; and the authors have found that, excluding insects breeding in carcasses washed up on the shore, only five species—one of Diptera, two of Collembola and two of mites—have become established by forming breeding populations. Finally, an attempt is made to predict the changes which are likely to take place in the fauna and flora of Surtsey during the next century.

The book contains numerous tables, diagrams, maps and photographs. Most of these are clear and informative but some of the photographs lack definition and the scale of some of the maps is rather too small. It is rather difficult to find one's way about the book owing to lack of clear distinction in the hierarchy of subheadings: centring of the earlier subheadings with the use of more distinctive lettering would have made the book easier to read.

But these small defects in presentation are relatively unimportant and Dr Lindroth and his co-authors are to be congratulated on producing an invaluable record of the initial stages of this unique natural experiment: the Surtsey Biology Conference was fortunate in its choice of such an able team. And owing to the recent devastating eruptions on Heimaey the detailed investigation of its fauna, though incidental to the main study, has its own special value.

E. H. EASON

Primates compared

An Atlas of Primate Gross Anatomy. By Daris R. Swindler and Charles D. Wood. Pp. 370. (University of Washington Press: Seattle and London, August 1973.) \$30.

THE maturation of comparative primate biology as an academic field during the past fifteen years has seen a resurgence of interest in primate morphology. Such study has again become an active research field as well as a basis for the functional appreciation of primate fos-

sils. In this context, much emphasis now attaches to the quantitative definition of variable morphological features that are functionally related to aspects of behaviour such as locomotion. Correspondingly, undergraduate training in physical anthropology includes instruction in the relevant basic comparative morphology.

Texts in this field suitable for use with honours students are not extensive and comprise, in general, accounts of only a single genus. This volume takes a welcome step in providing textual notes supplementing an extensive series of illustrations of high quality, grouped to form 149 plates. The whole is well set out to facilitate comparison between three primate genera: baboon (*Papio*), chimpanzee (*Pan*) and man (*Homo*). *Papio* and *Pan* were chosen for comparison with man because of their wide use in biological and clinical research and because they "... represent very different grades of locomotor adaptations" (*Papio*—a terrestrial quadruped; *Pan*—a brachiator, with *Homo*—a biped). Illustrations for the two subhuman genera are based on a series of dissections of twenty-two baboons and six chimpanzees; those for man are composite from numerous published sources.

The approach is regional although there are some allusions to systemic study. The result is a compilation which even although textually brief presents an unparalleled series of well drawn comparative figures. Each is intended to represent the norm of the series examined, certain inserts referring to principal variations.

Although proper attention is drawn to conspicuous topographical and proportional differences between the genera, the main impact of the text is to emphasise their basic similarity: this characteristic may well enhance the value of the volume as a student's text. Its potential worth as a reference monograph is, however, lessened by the fact that, despite the author's emphasis in the introductory sections upon the value of a proper appreciation of variation, its documentation is inadequate. Variation within and between these genera is marked; in some features, it can be readily defined as meristic; in others it is continuous and it is in relation to these that significance emerges in connection with functional contrasts.

The series of dissections on which the volume is based is, in many ways, unique, and the inclusion of relevant

numerical data, even as an appendix, would give value as a research work to a volume which, in its present form, is basically only a teaching manual.

E. H. ASHTON

Ancient invertebrates

Paleobiology of the Invertebrates: Data Retrieval from the Fossil Record. By Paul Tasch. Pp. xxv+946. (Wiley: New York and London, August 1973.) £10.

THIS book is intended as an advanced textbook of invertebrate palaeontology, and is a very ambitious undertaking for a single author. It is comprehensive, detailed and profusely illustrated. Unfortunately, however, it gives the impression of having been written much too quickly for its thickness. Altogether it is a most exasperating book, despite its compendiousness, and it is not worth the price.

There are countless spelling mistakes. These are not trivial since, after all, zoological names need to differ by only one letter to be validly distinct. Thus, on page 159 alone, *Aulacophyllum* is written as *Aulocophyllum*, *Phillipsastraea* as *Phillipastrea* and *Zaphrentis* as *Zaphrenthis*.

More serious are errors of fact. Nemertean are never parasitic (contrast page 445). There is no reason why a type subspecies need occur earlier than a non-typical subspecies (page 853). Pogonophora are not a genus of hemichordate (page 810) and ascidians are not hemichordates either (page 635). The anal pyramid of eocrinoids had to do with defaecation, but this is not the same as excretion (page 709). The segments of an ophiuroid's arms are not the same thing as its vertebrae (page 736). The word "asterozoan", contrary to the muddled discussion on page 736, is not synonymous with "asteroid". Hedgehogs, indeed, have never been included in the echinoderms (page 631). Most of these mistakes are slips of the pen or due to stylistic slovenliness, but they have been printed nevertheless, and wait in ambush for the student.

The author has a pretentious habit of using 'cybernetic' terminology whenever he sees the opportunity: the questions at the end of each chapter are called "feedback", the bibliography is called "storage", the anatomy of a group comes under "encoded data" and its stratigraphy and palaeoecology under "derivative data". The advantages of this procedure escape me. On the other hand, such necessary words as "haemocoel" (page 313), "lobe" and "saddle" (page 386) and "metapinnule" (page 739) are used without explanation of what they mean.

There are several banal discussions on problems that are insoluble in principle,

such as why groups died out. The last of the arthropleurids apparently became extinct "probably through failure to yield adequate numbers of viable offspring" (page 606). Parablattoids and edrioblastoids died out from a "continual decrease in the number of reproducing individuals" (page 725). But echinoids are ready for a great expansion in the future (page 695).

Concerning the index, I tried to look up the word '*Halycystus*', used on page 127, suspecting it might be identical with '*Halicystus*', which happens to be the correct spelling, on page 129. After some search I found it, with the wrong spelling, under 'S' for 'Scyphozoa'. Is this the data retrieval referred to in the title?

It may be that a new advanced textbook of invertebrate palaeontology is now needed, but this book is not the one.

R. P. S. JEFFERIES

Glass

Glass Science. By Robert H. Doremus. (Series on the Science and Technology of Materials.) Pp. viii+349. (Wiley-Interscience: New York and London, August 1973.) £9.

THIS is an authoritative well written book in an area where relatively few books are available. The dust cover note says that this book "will be used as a text for advanced undergraduate and graduate level courses in glass science and by technologists of diverse backgrounds who are involved in the manufacture and use of glass in industry". The author himself states that his object is to describe the state of glass science in 1972, a much more satisfactory description of the scope of the book if one interprets the title to mean "the physics and chemistry of glasses". The title has some unfortunate suggestion of a special kind of science, a comment which the author tends to invite by his generosity in quoting opinions and hypotheses.

Readers of the book are expected to be conversant, for example, with X-ray diffraction and its interpretation by means of pair distribution functions, the Mössbauer effect, NMR, infrared and Raman spectroscopy, and solution thermodynamics. A copy of Morey's well-known book *Physical Properties of Glass*, long since out of print, unfortunately, is assumed to be available.

The book contains five parts; 100 pages on the formation and structure of glasses, 113 pages on transport processes of which 80 deal with diffusion and electrical conduction, 60 pages on chemical and surface properties, 40 on strength and 14 on optical properties. The choice of subjects reflects the author's own interests. Each of the eighteen chapters has on average sixty

references to original papers so the book is an excellent entry to the literature. The author has succeeded in his aim of reviewing the information available in 1972.

The book will be especially useful to people who have recently taken up work in this field. It should certainly be on the reading list for advanced students of glasses.

R. W. DOUGLAS

Biological bouchées

Cell Differentiation. By J. M. Ashworth. Pp. 64. *Cellular Development.* By D. R. Garrod. Pp. 64. *Functions of Biological Membranes.* By M. Davies. Pp. 62. *Biochemical Genetics.* By R. A. Woods. Pp. 64. (Outline Studies in Biology.) (Chapman and Hall: London; Halsted (Wiley): New York, May 1973.) £0.90 each.

THE breakdown of the barriers between biological disciplines in university teaching has made it difficult to find adequate textbooks, for few reflect the broader approach now appropriate. A similar difficulty exists in providing suitable reading material for the increasing number of students who combine a variety of shorter courses in some form of integrated degree course. This new series of readers, each containing the equivalent of a short lecture course on a selected topic, could make a valuable contribution towards solving some of these difficulties and is therefore extremely welcome.

Ashworth, on *Cell Differentiation*, has selected a set of model systems that illustrate increasing levels of complexity from prokaryotic and eukaryotic organisms, and has shown how the basic behaviour can be analysed by genetic and biochemical techniques. He also gives an account of the characterisation (mainly biochemical) of such classic systems as chromosome structure in *Drosophila*, RNA synthesis in *Xenopus* and protein metabolism in different tissues, covering a wide range of experimental approaches. Ashworth contrasts the total analysis that is apparently possible in the simpler systems with the approach to these classic systems in which some exaggerated feature is studied. This conveys very clearly the decisions that have to be made by the intending research worker.

Garrod's book on *Cellular Development* complements the first book. He concentrates on pattern formation, and includes slime moulds (already discussed by Ashworth), hydra, limb bud formation, insect cuticle, nerve connections and embryonic systems. Simple models suggestively mimic the patterns of development, but the lesson that comes across is that these models are too

remote at the moment to shed much light on the molecular basis of development. This basis may be quite subtle and complex, but the challenge is irresistible.

Davies, in *Functions of Biological Membranes*, has made the physico-chemical basis of membrane function the central theme, with emphasis on the well studied membrane systems in higher organisms. Gradients (chemical, electrical and osmotic) underlie all significant membrane functions, and have defined characteristics under steady state conditions; these properties certainly need, and here receive, clear exposition. I hope, however, that there will be a companion volume in this series to deal with the nature of the carriers that lend selectivity to transport processes and that couple different transport processes to each other and to a source of energy. Both membrane and transport mutants are the focus of considerable effort in the bacterial systems; Davies could have given clearer pointers to their potential.

Woods has produced a slightly longer reader in *Biochemical Genetics*, selecting and compressing a considerable amount of material in the process. It requires some degree of concentration to follow, for instance when material from prokaryotic and eukaryotic systems is juxtaposed. The book is, however, intended for students who already have some background in genetics. Selection also generates problems—for instance, when the structure of nucleic acids is considered, the melting and annealing properties of DNA are omitted. These properties both illustrate the origin and stability of the double helical structures and underlie the methods for assessment of specific nucleic acid regions or species present in any preparation (including messenger RNA measurements, which give evidence for the transcriptional level of regulation and hybridisation to determine the nature and extent of repetitive genes in eukaryotic cells). The nature of the genetic code is perhaps the most complete section; others on mutants and metabolism and the genetic regulation of metabolism (the systems for lactose and arabinose utilisation and tryptophan production in *Escherichia coli*) are brief. Polar effects of mutations (strongly indicative of operons) are not discussed; this is certain to create some difficulty for the student. The I gene in the *lac* system is not part of the *lac* operon, though both Woods and Ashworth suggest it is.

Written in such a short space, these texts must be compromises, and with only relatively minor points of issue they can be regarded as successful. If the editing can ensure internal cross referencing and consistency, the coverage as the series develops could be extremely useful. There may be many potential authors who already teach

suitable material in course form who would be prepared to write at this length, so that it should be possible to maintain the standard already set.

J. F. COLLINS

Human guinea pigs

Human Guinea-Pigs. By Kenneth Mellanby. Second edition. Pp. 200. (Merlin: London, October 1973.) £2 boards; 80p paper.

THE parasitic mite *Sarcoptes scabiei* var. *hominis*, about one sixtieth of an inch long, burrows in the outermost layer of the epidermis. Scabies, the resulting condition of the host, is characterised by intractable itching which, after about three months, becomes severe enough to prevent sleep and, through consequent scratching, to cause sepsis in the skin. Scabies can be an important cause of absence from school and has been a matter of serious concern in the army.

Dr Mellanby describes his experiments on scabies during the Second World War. The mites were believed to be transmitted by bedding and clothing, which was therefore stove to prevent transmission of the disease. What was not clear was how long the mite could live in the bedding. With forty-seven conscientious objectors, who had volunteered for the work, Dr Mellanby set up a research institute in a Sheffield house to study the mite and the disease. Despite the boredom when without the disease, and the itch and pain when with it, the volunteers loyally and intelligently collaborated to the conclusion of the experiments. It was found that scabies was spread by direct contact, that bedding was not important in transmission, and that two days in a warm airing cupboard sufficed to kill the mite. Thus research costing under £5,000, once and for all, was able to save millions of pounds a year otherwise spent by local authorities on stoving. The volunteers were also subjects for dietetic experiments on calcium balance and, after the scabies work was concluded, remained as a unit under the Medical Research Council for experiments on lack of vitamins A and D. Altogether the work of the human guinea pigs lasted about six years.

This book was first published in 1945 and in this 1973 edition Dr Mellanby has added chapters on the vitamin studies, and on Sir Neil Hamilton Fairley's work with malaria prophylaxis in human volunteers, by which the malaria rate among Australian soldiers in New Guinea was reduced from 740 per thousand in 1943 to an annual rate of twenty-six per thousand in November 1944. Dr Mellanby has also added a chapter in which

he extracts from the German concentration camp experiments whatever could be of value, which, as he writes, "make up only a tiny fraction of the atrocities committed ostensibly in the name of medical research".

Dr Mellanby's experience with volunteers lead him to the general conclusions that an institute of human biology, for which subjects could volunteer, could be scientifically valuable; he points out that the Common Cold Unit is at present the only institute at which volunteers regularly serve. Just as he sees possibilities in work with "genuine volunteers, intelligent enough to understand the situation, and always free to terminate their contract", he is cautious whether the word 'volunteer' can ever be properly used for someone imprisoned.

Through the book we learn a little of the author, of the conscientious objectors, and of their understanding of each other, as it developed. Whether the topic is people, the practice of experiments or the author's general conclusions, the writing is always lucid, precise and easy to follow. The manner is modest, without exaggeration of the achievement or unjustifiably wide generalisation from results. This modesty adds force to the author's words when he gives his reasons for preparing the 1973 edition.

One of these is that this work on scabies, of obvious applicability and amply justifiable economically, could never have originated as a Rothschild 'customer-contractor' enterprise. "The work was only done because a very junior research worker thought that something should be done, and then put forward a hare-brained scheme which, miraculously, attracted a modest amount of support, even if . . . only . . . after he had crusaded with missionary zeal".

Finally one may, with Dr Mellanby, note that "the great Viennese dermatologist Hebra discovered almost all the main facts about scabies a hundred years ago, and if proper reliance had been placed on his results, a great deal of human suffering would have been avoided". This lesson is applicable in many fields.

JEFFREY BOSS

Words for minds

The Language of Psychoanalysis. By J. Laplanche and J.-B. Pontalis. Pp. xv + 510. (International Psychoanalytical Library Series.) (Hogarth Press and Institute of Psycho-Analysis: London, November 1973.) £6.50.

THE title of this book suggests that it is an enquiry into the nature of psycho-analytical language, rather in the way

this was recently undertaken by the sociologist Maurice Roche in his *Phenomenology, Language and the Social Sciences*. Such a book would have been welcome especially if written by someone with direct experience of psychoanalytic work. But the title is misleading: the book is a dictionary, and sets out to elucidate, in detail, the accepted meanings of the many concepts used in psychoanalysis, including an account of their evolution.

In a short introduction Daniel Lagache justifies psychoanalytical terminology, maintaining that "ordinary language has no words to evoke mental structures and tendencies that do not exist for common sense." But do mental structures of the kind proposed by Freud exist? Is there really an id? Surely it is the naive acceptance of such an idea that has led Freudian theory into much disrepute? The question as to how to describe "tendencies that do not exist for common sense" is more debatable. Freudian theory seems to have gone astray here too—but it is not alone in so doing, being in the company of traditional psychiatry and psychology and most if not all disciplines which have attempted a "scientific" account of human meaning and behaviour. The more the language departs from that of ordinary common sense the greater it removes itself from psychological reality and in the end we are lost in a mass of jargon and a complexity of tautologies.

Because the authors do not question Freud's basic intellectual stance the book is vulnerable to criticisms of this kind. It lacks the objectivity of Charles Rycroft's delightful but all too short *Critical Dictionary of Psychoanalysis*. But having expressed my dissatisfactions with the book I must confess to an admiration for it. It is clear and scholarly, avoids tautology as far as can be done in the context of the task and gives a tight discussion not only of the usage of terms but some of the relevant problems of psychoanalysis to which they refer.

PETER LOMAS

Cell membrane systems

Comparative Organellography of the Cytoplasm. By A. Frey-Wyssling. (Protoplasmatologia, Vol. III/G.) Pp. vii + 106. (Springer-Verlag: Vienna and New York, 1973.) 296 schillings; 42.40 DM; \$23.90.

THIS is a short account of the present state of knowledge about the membrane systems, microtubules and microfilaments of plant and animal cells. It is very condensed but because the subject matter is presented from such a definite

viewpoint, the ideas and theories are very stimulating. The generalisations do, however, in most cases need more reservations and qualifications than they are given. In this connection it is a pity that in such a short book space is given up to discussions of the Greek origins and other aspects of nomenclature rather than an amplification of conclusions.

Because of its condensed form and the bias that is given to the subject matter the book is rather unsuitable as background reading for elementary students. Most workers in the field will, however, find it very interesting, and although they may not agree with all the ideas the account does give an overall perspective that may well be lost by concentration on one particular aspect of this very important and exciting field of biology.

D. H. NORTHCOTE

Cinderella controllers

Control and Urban Planning. By J. Brian McLoughlin. Pp. 287. (Faber: London, October 1973.) £3.95.

BRIAN McLOUGHLIN draws together two threads in his latest book: his long standing interest in the theory of control, and the results of a research project on development control in planning. He discovered that the development controllers, planning's case-workers, feel themselves to be the Cinderellas of planning: the whizz kids go into 'strategy' or 'research'. And yet it can be argued, as it is here, that the essence of planning is control, that its main instrument is development control, and so the latter must be connected to the latest research in the field of the former.

The argument is set out as follows. An historical account of the nature and instruments of control in planning, including an effective review of official reports and government publications, is given in chapters 1-5. The implications of the 1968 Act, and structure planning, are taken up in chapter 6. The need for a more explicit treatment of policy making is identified and this is pursued in chapter 7. The theory of control and its application in urban planning, largely from the viewpoint of cybernetics (Ashby, Klir, Valach, and Beer loom large) is presented in chapters 8 and 9, and is connected to the issues for urban planning identified in earlier chapters. Some recommendations are presented in chapter 10.

What emerges from the argument? The early emphasis on development control leads to an exceptionally well balanced approach overall, as this aspect is usually neglected. It also

brings to the fore the need for a more explicit treatment of policy. General strategic statements are insufficient for the needs of the development controller. Considerable illumination is cast using the theory of control. Three examples will suffice. First, Beer's concepts of hierarchies in control systems, and associated differences in language between levels, show that there is an as yet unbridged gap between strategy and micro decision levels. Second, Ashby's law of requisite variety helps to determine necessary characteristics of the control system. Third, several levels of resolution are needed, and the two problems cited above will look different at different levels.

All of this will stimulate future research. One of the main roles of the book, however, will be to connect planners who have had a traditional education to these newer ideas, and such exposition has always been McLoughlin's forte. It is a happy accident that the argument is rooted in development control and the issues raised by the demands of structure planning, as the reader is immersed in this before being led gently into control theory. The myth that control theory leads to government by computer is laid bare. The traditional planner will almost be forced to recognise that the new insights will help, and indeed are necessary, for any underpinning value system. The 'new planning' is clearly seen as an aid to the political process and not a substitute for it.

It is possible that a few people will be put off by cybernetic jargon, and it remains an interesting speculation that although this presentation is sufficient, it may not be necessary! There may be other perspectives from which some of the new ideas can be explained to a wider public. It is also possible that the cybernetic viewpoint sometimes forces a view of urban systems as such (as distinct from associated planning processes) which overemphasises, say, homeostatic adjustment, relative to other systems analytical approaches—such as statistical averaging or accounting procedures. But in relation to the main purposes of the book these criticisms are minor. Its arrival is most timely. Urban planners are attempting to be more scientific. The traditional concerns of physical planning are having to be integrated with many other fields of urban government into systems of government. New local authorities take over from the old in April 1974. The author provides an excellent agenda of the issues facing them and many insights towards solutions to problems. The book is well written, easy to read and deserves a wide audience.

A. G. WILSON

science on radio

Burning issues

John Hall

EVEN when we are in the eye of a storm as far as energy resources are concerned, a measure of the seriousness which the BBC attaches to the subject may be gauged from the time slot it allowed Brian J. Ford's assessment of possible solutions, "Filling the Energy Gap", in the series "Where Are You Taking Us?". Radio Four at 10.15 on a Sunday evening is not exactly a prime spot to air a burning issue, especially when it puts a strain on one's loyalties to Monty Python's Flying Circus, which is pitched at a level of concern more appropriate to the tail end of the two-day break. Maybe the non-alarmist conclusion of the programme played a part in its placing; broadly, after examining the evidence, Ford could not forbear remarking that perhaps the Arab induced energy problem was a blessing in disguise. Better to be reminded of the urgent need for energy research by a merely political shut-down than by an actual, *bona fide* exhaustion of the oil, he argued. Which of course is an eminently defensible world view of the situation, though less satisfying as an answer to a Swedish motorist, or a Dutch polythene manufacturer, to whom the distinction between actual and political scarcity must appear academic.

Taking the supra-national view then, the programme pointed up the fact that there is more energy lying around than we know how to use, and once we can shake off an addiction to the destruction by burning of fossil fuels, the sooner we are likely to happen on the technological keys to a hydrogen fusion and fast breeder power strategy.

Evidence for these conclusions was offered by Sir Peter Kent, chairman of the Natural Environment Research Council, and Dr Ian Fell, a fuel technologist working at Newcastle University. The historical basis for the present pretty pass, both men agreed, was that the past cheapness of oil had rendered alternative energy propositions so economically unviable that they had not been adequately researched. A four-fold rise in crude oil prices in one year altered the situation somewhat.

Dr Fell's hopes for the future centred on electrical energy from nuclear fusion; given the technology to contain high

temperature plasma, nuclear fusion offered the prospect of all eternity without a worry about energy. Less spectacularly, at least according to present materials costs, the fuel cell might offer a good means of powering traction, given successful research. The Sun, in fact, was held out as the ultimate provider. According to Sir Peter the energy involved in sinking a shaft to tap geothermal sources might require a greater input than could be extracted; and Dr Fell envisaged an expenditure of £1,000 million on a Severn barrage scheme providing 10% of present British electrical requirements. But, free of charge and by courtesy of photosynthesis, solar energy produced an annual equivalent of 100 million tons of coal, largely in the forests of the Third World.

Sir Peter looked for research into ways of taking energy from coal deposits without actually bringing the stuff up in tubs, and Dr Fell asked for work in the pure physics and chemistry of burning, a basic part of energy conversion presently receiving scant attention. Fundamental research apart, simply finding out how to adjust a furnace already in use might effect a saving of 10% to 20%. And on the subject of making the best use of the tools at our disposal, Dr Fell was even hopeful that the current crisis might focus the minds of our politicians wonderfully, so that we should be enabled to emerge, after two or three difficult years, unbowed, if not unbloodied.

One small step

John Gribbin

It is often said that the best gamekeepers are reformed poachers. On that basis, the choice of John Maddox to present BBC Radio 3's new fortnightly programme "Scientifically Speaking" seemed ideal, as anyone who was a regular reader of *Nature* a year or so ago will appreciate. But unfortunately the possibilities raised by this choice were left unfulfilled, in the first programme at least, and Maddox was heard to be cautiously feeling his way, making one small step forward rather than taking a giant leap into the new lands of broadcasting.

The start of the programme promised well, with Maddox putting over his own

view and asking why science should not be popular and whether it is sensible to starve research of funds, and commenting on the passive way the research councils toe the line of government restrictions. Maddox riding these hobby horses is worth listening to and makes a lot of sense. But when it came to putting over recent advances in science, rather than commenting on the politics of science, one of the questions was answered: science lacks popularity, to some extent at least, because it is put over in such a dull way by programmes such as this.

Science is fun, as Maddox is fond of saying. But you would never guess it from this programme. I can find no fault with the choice of subjects (spreading of the Red Sea, Professor R. A. Lyttleton's view on comets, hepatitis B and the possibility of determining the sex of children in advance). Even these exciting subjects, however, came over in the usual interviewer/interviewee format, full of indigestible facts. Maddox is not the best interviewer in the world, and the indigestibility of the facts was compounded by his own difficulties, especially with Lyttleton, who allowed the questioner little use of the microphone.

Comet Kohoutek passed some 7 to 8 million miles from the Sun, said Maddox; actually no, more like 13 million miles, corrected Lyttleton. But, he added, there was one comet that came within a hundred thousand kilometres. Halley's Comet was mentioned—due in 1996 (Maddox) or 1986 (Lyttleton) depending on how carefully you were listening. And summing up this discussion Maddox told us that a space probe sent to the tail of a comet would resolve the dust cloud/icy nucleus argument; wrong again (although this time Lyttleton was not present to correct the error); the probe should, of course, be sent to a comet's nucleus.

The story of choosing a baby's sex offered plenty of scope for fun, none of which was taken up. More interest would have been aroused, surely, by reporting the folk-lore tale that pilots of jet fighters tend chiefly to have daughters, because of the effect of high *g* on sperm, and asking the experts to account for that in terms of the recent Schering work.

These may be the subjects about which scientists are excited, but you

would never guess so from this kind of interview. Maddox is at his best as a commentator and all that this programme needs to become really worthwhile is to use him in that role

while unloading some of the interviewing donkey work on to someone more adept at the task. If the powers that be at the BBC could then be persuaded to ensure that it did not clash with the

only regular science programme on television ("Horizon") those of us who have been lobbying for more science programmes would really have something to shout about.

obituary

Sir Robert Waston-Watt

SIR Robert Watson-Watt, pioneer of radar, died on December 5, 1973. He was 81 years old and had been in poor health for some time.

He obtained his early education in Brechin, his birthplace, and proceeded with a bursary to University of St Andrews where, at University College, Dundee, he studied electrical engineering. After graduation he was appointed Assistant to the Professor of Natural Philosophy at Dundee, a post which he occupied until 1915 when he became an assistant at the Branch Meteorological Office at the Royal Aircraft Factory, Farnborough.

It was during his service at Farnborough that he took up the study of thunderstorms by means of the radio waves, or atmospherics, emitted by them. After the end of hostilities, work on atmospherics and their relation to meteorological phenomena became the main investigation of the station of which he had been made Meteorologist-in-Charge in 1917. He continued in charge of the station after its transfer to the Department of Scientific and Industrial Research in 1921 and its removal to Slough in 1924. In 1927 he became Superintendent of the Radio Research Station set up to absorb the atmospherics work and other radio research of the Department of Scientific and Industrial Research in progress at Slough.

In his early studies of atmospherics one of the main problems was the location of their sources. Although some success on streams of atmospherics was obtained using Bellini-Tosi direction finders, it was Watson-Watt's invention of the instantaneous visual cathode-ray direction finder (CRDF) which enabled the source of a single atmospheric to be found and greatly facilitated the whole investigation.

During the Second World War Watson-Watt's CRDF apparatus was operated by the Meteorological Office as a means of observing the positions of thunderstorms around the British Isles in areas from which no synoptic reports

were then received. Similar equipment is still regularly used by the Meteorological Office to help in the production of weather forecasts.

The ability of the CRDF to work on short duration signals found an important application during the Battle of the Atlantic during which ship- and shore-based apparatus was used to locate U-boats from bearings obtained on their brief radio transmissions.

Watson-Watt will, however, be best remembered for his pioneering work in radar. This began early in 1935 when he was approached informally by the Air Ministry for an independent appreciation of the possibility of generating a radio 'death-ray' for use against enemy aircraft. While rejecting the feasibility of such a ray, he reported that the detection and location of aircraft at useful ranges was possible and subsequently submitted a memorandum giving his proposals on how this could be done to Sir Henry Tizard's Committee for the Scientific Survey of Air Defence. After a successful demonstration of the underlying principles he was requested to begin development work along the lines indicated in the memorandum. This he did at Orfordness where the work prospered rapidly and was soon moved to Bawdsey, near Felixstowe.

Appointed Superintendent of Bawdsey Research Station in August 1936 he and his team devoted themselves energetically to those forms of radar, both ground-based and airborne, which were to play such a vital part in the Second World War.

After the successful initiation of radar, Watson-Watt, while continuing to make important technical contributions, found his main activity in cogent advocacy of radar in Whitehall and in stimulating the bureaucratic machine into unwonted activity to provide the material requirements of the research worker and of the coastal stations. These activities led, in 1938, to his appointment as Director of Communications Development, Air Ministry, but soon after the outbreak of war, his talents found more congenial use as

Scientific Advisor on Telecommunications at both the Air Ministry and Ministry of Aircraft Production, and as Vice Chairman of the Radio Board of the War Cabinet.

After the war he retired from Government service to conduct a consultancy business, mainly in North America, which he set up with wartime colleagues.

Amongst the many honours he received were C. B. and Knight Bachelor; he was a Fellow and Hughes Medallist of the Royal Society and held honorary doctorates of St Andrews, Toronto and Laval Universities. He was a Past President of the Royal Meteorological Society and of the Institute of Navigation.

A man of great gifts and wide interests he was a genial, courteous and well-informed companion.

Sir Ronald Holroyd

SIR Ronald Holroyd, FRS, who died on September 29, was an outstanding industrial scientist. His genius lay not in success as an individual worker, but in a capacity to organise large-scale research projects that were the foundation of important new processes. His field was coal and petroleum chemistry, and he was responsible for developments which were of major national importance during and immediately after the second world war.

Holroyd was born in Yorkshire on April 26, 1904. After going to Holgate Grammar School, Barnsley, he read chemistry at Sheffield University (1921-25). After a short period with the Board of Trade, he joined ICI in 1928. Originally with Brunner, Mond at Winnington, he transferred to Billingham in 1932. In 1947 he was appointed research director of Billingham Division, and five years later joined the ICI Main Board in London, quickly becoming research director (1935). In 1957 he was appointed a Deputy Chairman, and held this position until he retired in 1967.

At Billingham, Holroyd was partic-

ularly concerned with the production of liquid hydrocarbons, including petrol, by the hydrogenation of coal and creosote, and a plant was put into operation in 1935. The outbreak of war in 1939 gave new urgency to this process, especially for the supply of aviation fuel, because of the lack of petroleum-refining capacity in Britain. In 1941 a consortium of ICI, Shell, and Trinidad Leaseholds set up a gas-oil hydrogenation plant at Heysham, and in the latter years of the war some half million tons of aviation base spirit was produced in Britain annually. Among technical problems overcome was that of increas-

ing the octane rating, partly by increasing the aromatic content and partly by additives such as tertiary butyl benzene (Victane) and monomethyl aniline. This was of crucial importance in enabling Spitfires to catch German V 1 rockets. Apart from their intrinsic importance, these developments resulted in an accumulation of knowledge and experience of hydrocarbon chemistry, which was invaluable in the post-war chemical revolution when petroleum almost wholly superseded coal-tar as a raw material.

An off-shoot of this work was the development of analytical techniques for hydrocarbons, especially infra-red and

ultraviolet spectroscopy. Analysis of fuel from shot-down enemy aircraft threw much light on the German oil supply situation. In the closing stages of the war, Holroyd led several teams which went to Germany to investigate oil and aviation fuel developments there.

Holroyd's contributions were widely recognised. He was elected Fellow of the Royal Society in 1960, and was knighted in 1963. He was an honorary graduate of Oxford, Sheffield, Hull, and Trinity College Dublin. In 1958 he was awarded the Castner Medal of the Society of Chemical Industry, of which he was President 1965-7.

matters arising

Mesozoic rocks from the Labrador sea

BECAUSE of the great interest, both academic and commercial, in the samples reported by Johnson *et al.*¹, additional stratigraphic analyses were conducted in Pau, France, by Aquitaine Company of Canada (Table 1). These analyses were concentrated on the nannofossils; especially the coccoliths (G. Chennaux and S. Mulle, unpublished).

As noted by Chennaux and Mulle (Table 1) the samples show a heterogeneity of ages ranging from Mesozoic to Recent. The Mesozoic nannofossils are in a poor state of preservation in contrast to well preserved Tertiary nannofossils, which supports the younger age. The presence of Mesozoic spores and pollens¹ are therefore assumed to be re-worked organic debris deposited in the prograding Tertiary sequences.

A Tertiary age for these samples would be in agreement with seismic ve-

locities from the outer Greenland shelf (N. J. McMillan, personal communication). An Oligocene date plots along a more normal subsidence rate of 30-40 m per m.y. (ref. 1, Fig. 2).

These revised ages for Greenland samples suggest that the Mesozoic ages reported from the Labrador continental margin² may need revision too.

We thank J. Aubert, F. Deres, C. Boulovard, G. Peniguel, and C. Poumot all of Centre de Recherches de Societe Nationale des Petroles d'Aquitaine, Pau,

TABLE 1 Revised data on South-west Greenland dredge samples

Sample No.	Position	Age of associations		Age proposed by Johnson <i>et al.</i> ¹	
		Nannofossils	Palynoplanktology	Palynoplanktology	Revised age
32	60°04.8'N 46°44.0'W	Oligocene (and remains of Lower Cretaceous)	Lower Palaeozoic Triassic Cretaceous to Jurassic	Upper Cretaceous (and Jurassic remains)	Oligocene
37	60°04.6'N 47°10.5'W	Oligocene to Lower Miocene	Undetermined	Upper Cretaceous (Albian or younger) (and Jurassic remains)	Oligocene
43A	63°34.3'N 52°57.0'W	Oligocene	Probably Recent Lower Cretaceous	Upper Cretaceous	Oligocene
43B	63°34.3'N 52°57.0'W	Probably Eocene	Cretaceous? Triassic? Carboniferous?	Upper Cretaceous (Turonian—Senonian Lower) (and Jurassic and Upper Carboniferous relics)	Eocene
56	61°35.5'N 50°37.1'W	Eocene-Oligocene (and Jurassic remains)	Lower Cretaceous Recent? Triassic Lifeless	Upper Cretaceous (and Jurassic and Upper Carboniferous relics)	Eocene to Oligocene
59A	61°53.0'N 50°45.0'W	Middle Eocene to Oligocene		Upper Cenomanian to Lower Turonian	Oligocene
59B	61°53.0'N 50°45.0'W	Lifeless	Lower Cretaceous Triassic	Upper Cenomanian to Lower Turonian	
62A	62°35.0'N 51°35.0'W	Lifeless	Triassic to Cretaceous	Pliocene to Recent (and Cretaceous, Jurassic and Palaeozoic)	Pleistocene? to Recent
62B	62°35.0'N 51°35.0'W	Probably Recent	Recent? Lower Cretaceous Triassic	Turonian	Pleistocene? to Recent
65	63°07.5'N 52°17.8'W	Lower Palaeocene	Lower Cretaceous	Jurassic lower (Bajocian or Pliensbachian) and undetermined	Lower Palaeocene
69	64°25.0'N 52°53.0'W	Tertiary (and remains of Cretaceous)	Undetermined	Palaeocene (and Jurassic remains)	Palaeocene

France who conducted the biostratigraphic studies, and J. McMillan of Aquitaine Company of Canada who reviewed the manuscript.

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¹ Johnson, G. L., Campsie, J., Rasmussen, M., and Dittmer, F., *Nature phys. Sci.*, **236**, 86 (1972).

² McMillan, N. J., *Geol. Surv. Can. Pap.*, 71-73, 451 (1973).

Announcements

International Meetings

February 28, **The Role of Satellites in Sensing the Global Atmosphere and their Application in Weather Forecasting** (General Secretary of the Remote Sensing Society, Dr W. G. Collins, Reader in Remote Sensing, Remote Sensing Unit, University of Aston, Gosta Green, Birmingham, B47ET)

February 28-March 1, **7th International TNO Conference** (The Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, Netherlands)

Errata

In the News and Views article, "From Cyclic AMP to Cyclic GMP" (*Nature*, **246**, 186; 1973) reference was made to volume 3 of *Recent Advances in Cyclic Nucleotide Research* by Goldberg *et al.* This volume is actually entitled *Advances in Cyclic Nucleotide Research* and will be published by Raven Press.

In the review of the book *The Estimation of Animal Abundance and Related Parameters* by G. A. F. Seber (*Nature*, **246**, 536; 1973) the number of pages should have been given as 506, not 56.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

British Antarctic Survey. Scientific Reports, No. 68: Vegetation of the South Orkney Islands, with particular reference to Signy Island. By Dr. R. I. Lewis Smith. Pp. 124+6 plates. (London: British Antarctic Survey, Natural Environment Research Council, 1972.) £3.75 net. [110]

Social Science Research Council. Annual Report 1972/1973. Pp. 76. (London: HMSO, 1973.) 47p net. [410]

Bulletin of the British Museum (Natural History). Entomology. Vol. 28, No. 1: The Genus *Etiella* Zeller (Lepidoptera: Pyralidae)—a Zoogeographic and Taxonomic Study. By P. E. S. Whalley. Pp. 1-21+15 plates. £2.60. Vol. 28, No. 4: A Catalogue of the Family-Group and Genus-Group Names of the Gelechiidae, Helicopogonidae, Lecithoceridae and Symmocidae (Lepidoptera). By K. Sattler. Pp. 153-282. £4.70. Geology. Vol. 23, No. 4: Palaeozoic Coral Faunas from Venezuela, II. Devonian and Carboniferous Corals from the Sierra de Perija. By C. T. Scrutton. Pp. 221-281+10 plates. £4.35. Vol. 23, No. 5: *Prodeinotherium* from Gebel Zelten, Libya. By J. M. Harris. Pp. 283-348+5 plates. £2.85. Vol. 23, No. 6: Cripripes from the Upper Cretaceous of Alabama and Mississippi, Eastern Gulf Region, U.S.A. I. Palaeontology. By J. S. H. Collins. II. Geology. By F. F. Mellen. Pp. 381-388. £2.25. Vol. 23, No. 7: Feneestratic Bryozoa from the Viscan of County Fermanagh, Ireland. By R. Tavenor-Smith. Pp. 389-493+26 plates. £7. Historical Series. Vol. 4, No. 4: The Entomological Publications of Pierre Millière (1811-1887). By K. Sattler and W. G. Tremewan. Pp. 221-280+3 plates. £2.70. Mineralogy. Vol. 2, No. 6: The Pseudoleucite Borolanites and Associated Rocks of the South-Eastern Tract of the Borralan Complex, Scotland. By A. R. Woolley. Pp. 285-333+6 plates. £2.95. Vol. 2, No. 7: Rocks from Antarctica—the Discovery Collection in the British Museum (Natural History). By D. R. C. Kempe. Pp. 335-376+7 plates. £2.45. Zoology. Vol. 25, No. 1: On the Cichlid Fishes of the Genus *Pelmatochromis* with Proposal of a New Genus for *P. Congicus*; on the Relationship Between *Pelmatochromis* and *Tilapia* and the Recognition of *Sarotherodon* as a Distinct Genus; and A New Species of Cichlid Fishes of Rivers Quanza and Bengo, Angola, with a List of the Known Cichlidae of these Rivers and a Note on *Pseudocrenilabrus natalensis* Fowler. By E. Trewavas. Pp. 1-37+1 plate. £1.65. Vol. 25, No. 2: Freelifing Marine Nematodes from the Indian Ocean. By R. M. Warwick. Pp. 85-117. £1.50. Vol. 25, No. 4: Biology and Fine Structure of *Euglypha rotunda* (Testacea: Protozoa). By R. H. Hedley and C. G. Ogden. Pp. 119-137+7 plates. £1.60. Vol. 25, No. 5: A Revision of the *Haplochromis* and Related Species (Pisces: Cichlidae) from Lake George, Uganda. By P. H. Greenwood. Pp. 139-242. £4.55. Vol. 25, No. 6: Preliminary Notes on the Ontogeny of the Frontal Body Wall in the Acanthaceae and Acanthellidae (Bryozoa, Cheilostomata). By P. L. Cook. Pp. 243-263+3 plates. £1.45. Vol. 25, No. 7: Some New Taxa of Recent Stalked Crinoidea. By A. M. Clark. Pp. 265-288+2 plates. £1.50. (London: British Museum (Natural History), 1973.) [1010]

Proceedings of the Royal Irish Academy. Vol. 73, Section B, No. 12: Pleistocene Glacial, Glaciomarine and Associated Deposits of Mell and Tullyallen Townlands, Near Drogheda, Eastern Ireland. By E. A. Colhoun and A. M. McCabe. Pp. 165-2-6+ plates 11-14. (Dublin: Royal Irish Academy, 1973.) 64p. [1610]

Memoirs of the Royal Astronomical Society. Vol. 77, Part 5: Radial Velocities of Southern B Stars Determined at the Radcliffe Observatory—VII. By A. D. Thackeray, S. B. Tritton and E. N. Walker. Pp. 203-222. (Oxford and London: Blackwell Scientific Publications, 1973. Published for the Royal Astronomical Society.) [1710]

People. Vol. 1, No. 1, October 1973. Published quarterly. Pp. 1-48. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1973.) [1710]

Smithsonian Contributions to Zoology, No. 141: Morphology of *Cypretta kawatai* Sohn and Kornicker, 1972 (Crustacea, Ostracoda), with a Discussion of the Genus. By I. G. Sohn and Louis S. Kornicker. Pp. 28. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 60 cents. [1110]

United States Department of the Interior: Geological Survey. Professional Paper 433-M: Relations Among Radionuclide Content and Physical, Chemical, and Mineral Characteristics of Columbia River Sediments. By J. L. Glenn. With a Section on Sand and Gravel Mineralogy by R. O. Van Atta. Pp. iii+52. 85 cents. Professional Paper 751-B: Design and Operation of the Artificial-Recharge Plant at Bay Park, New York. By Ellis Koch, Anthony A. Giaimo and Dennis J. Sulam. Pp. iii+14+plate 1. 95 cents. Professional Paper 754-A: Glacial and Postglacial Geologic History of Isle Royale National Park, Michigan. By N. King Huber. Pp. iii+15+plate 1. \$1. Professional Paper 809-A: Recognition of Natural Brine by Electrical Soundings Near the Salt Fork of the Brazos River, Kent and Stonewall Counties, Texas. By Adel A. R. Zohdy and Dallas B. Jackson. Pp. iii+14. 40 cents. (Washington, DC: Government Printing Office, 1973.) [210]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2028: A National Study of the Streamflow Data-Collection Program. By Manuel A. Benson and Rolland W. Carter. Pp. iv+44. 45 cents. Bulletin 1309: Chemical Analysis of Thermal Waters in Yellowstone Park, Wyoming, 1960-65. By J. J. Rowe, R. C. Fournier and G. W. Morey. Pp. iii+31. 35 cents. Professional Paper 790: Stratigraphy, Morphology, and Paleogeology of a Fossil Peccary Herd from Western Kentucky. By Warren I. Finch, Frank C. Whitmore, Jr., and John D. Sims. Pp. iii+25. 70 cents. Professional Paper 712-B: Geohydrology of the Eastern Part of Pahute Mesa: Nevada Test Site, Nye County, Nevada. By Richard K. Blankenbiller and J. E. Weir, Jr. Pp. iv+35+plates 1-3. \$1.75. Professional Paper 685: Fossils from the Ordovician Bioherm at Meiklejohn Peak, Nevada. By Reuben James Ross, Jr. Pp. iv+47+plates 1-18. \$1.25. Professional Paper 663-B: Structural Geology of the Sun River Canyon and Adjacent Areas, Northwestern Montana. By Melville R. Mudge. Pp. vi+52+plates 1-5. (Washington, DC: Government Printing Office, 1972 and 1973.) [310]

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CERN—European Organisation for Nuclear Research. CERN 73-11: High-Accuracy Measurements of the Centre of Gravity of Avalanches in Proportional Chambers. By G. Charpak, A. Jeavons, F. Sauli and R. Stubbs. Pp. 4. (Geneva: CERN, 1973.) [1610]

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